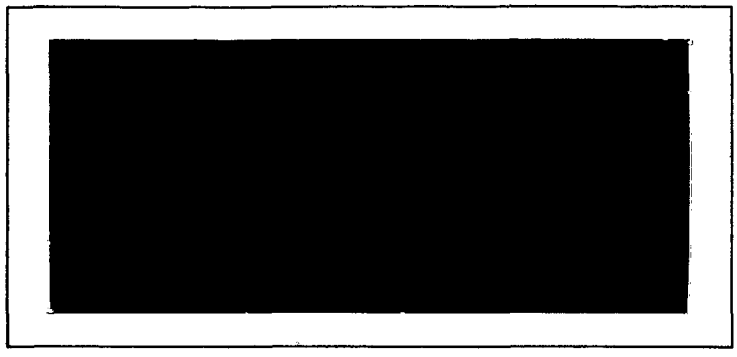

N72-10273 (NASA-CR-123293) STUDY OF THE POSSIBLE ROLE OF POLLUTION IN THE PREVALENCE OF SEA NETTLES IN THE CHESAPEAKE BAY AND THE DEVELOPMENT OF A CENSUS (Biospherics, Inc.)
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Report

136p.

Study of the Possible Role of Pollution in the Prevalence of Sea Nettles in the Chesapeake Bay and the Development of a Census Taking Method

Contract No. NASW-2115

FINAL REPORT

Prepared for:

National Aeronautics and Space Administration
Headquarters
Washington, D. C. 20546

Prepared by:

BIOSPHERICS INCORPORATED
4928 Wyaconda Road
Rockville, Maryland 20852

15 October 1971

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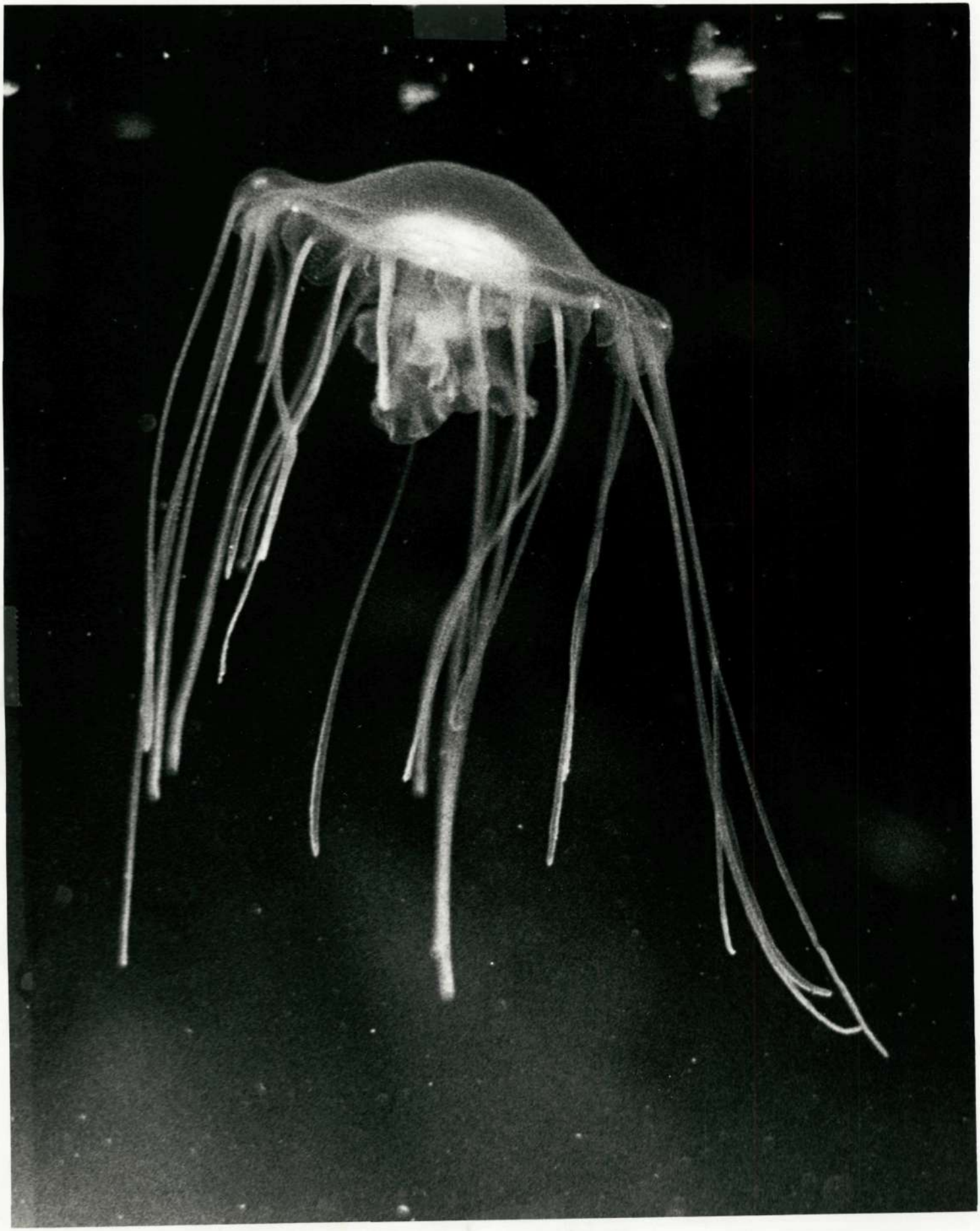
Frontispiece:

Chrysaora quinquecirrha Medusae



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We wish to acknowledge the valuable contributions of Miss Deborah Schuhmann and Mr. Edward Rich to the pollution and remote sensing aspects, respectively, of this program.

The helpfulness of the personnel at the Virginia Institute of Marine Science, especially Dr. Ken Webb and Miss Janet Olmon who supplied us with our stock polyp collection and with Cyanae medusae during the winter, is also appreciated. They spent many valuable hours teaching us morphological features and maintenance of polyps and breeding of Chrysaora medusae.

We also wish to thank Mr. Jim White at Photo Science for supplying us with GAF two-layer film and for discussing with us advantages and limitations of a variety of remote sensing techniques.

The cooperation of Dr. Frank S. Kennedy at the Florida Department of Natural Resources in St. Petersburg, Florida, who shared progress reports with us regarding his remote sensing program of the Portuguese man-of-war is also acknowledged.

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ABSTRACT

During our nine-month study, we have studied the sea nettle, Chrysaora quinquecirrha, to determine the effects of pollutants on the polyp stage, and to explore means to detect the medusae form by remote sensing.

To permit the remote sensing studies, sea nettle medusae were maintained for as long as ten weeks by keeping them in an uncrowded aerated aquarium and supplying them with fresh Chesapeake Bay water weekly. Although they appear to feed on tropical fish food and ctenophores, they, nonetheless, underwent severe shrinkage during this time and were unrecognizable as medusae after ten weeks. Polyps were more easily kept in finger bowls containing 20‰ artificial sea water. By changing the water weekly and feeding Artemia brine shrimp, polyps could be kept indefinitely, permitting relatively long-term studies on pollutant effects. Although polyps naturally multiply, it was found that the most effective way of obtaining high numbers was to sever polyps close to the tentacles. Both portions regenerate within a few weeks with almost 100% recovery.

In our pollution studies, we examined the effects of phosphate, nitrate, ammonium, combinations of these pollutants,

and synthetic sewage effluent on maintenance and morphology of sea nettle polyps. In addition, the effect of pH from 6 - 8 was examined. Several polyps were maintained in various concentrations of these pollutants at each of three temperatures (5°, 20°, 26°C) for several months and observed for morphological effects. The results of these studies indicate no effect of pH whereas phosphate and nitrate and combinations thereof may contribute to the proliferation of polyps. On the other hand, ammonium, combinations of pollutants (ammonium plus nitrate) and sewage effluent are detrimental to polyps and may cause their demise. It is of interest that phosphate and nitrate appear to afford protection against the lethal effects of ammonium. These effects were more pronounced at 26° than at 20° whereas at 5° all polyps encysted and no effects of pollutants were apparent. Concurrent with these findings, it was also established that, at high temperatures, polyps cause a sharp decrease in the ammonium concentration of the maintenance medium whereas the phosphate and nitrate concentrations are relatively unaffected by the presence of polyps.

When the temperature was raised from 20° to 26°, polyps in phosphate and nitrate began to multiply at about the same rates as did those polyps maintained constantly at 26°. Of the polyps maintained as cysts at 5° for several months, about 10-20% of the controls began to form polyps (some of which

strobilated) following the temperature rise to 26°. Polyps also strobilated following the temperature raises although strobilation was asynchronous even among pollution-free controls and of sufficiently low frequency to obliterate the detection of any pollution effects. Phosphate and nitrate had little effect on either low temperature survival or strobilation frequency whereas ammonium, combinations of pollutants, and sewage effluent prevented polyp formation.

To complement these studies, an assay based on the labeled release technique was developed for measuring polyp metabolism. Polyps were placed in 20‰ artificial sea water, antibiotics (to eliminate the interfering bacterial response), and ^{14}C-labeled organic substrates. They were then monitored for $^{14}CO_2$ evolution. Careful study determined that the most effective antibiotic was a combination of penicillin and polymyxin. The most effective substrate was a combination of ^{14}C-glucose and ^{14}C-alanine. Using this technique, it was shown that nitrate had no effect on polyp metabolism whereas ammonium was strongly inhibitory. Phosphate was slightly inhibitory although the inhibition was not directly related to the phosphate concentration in the range studied. Thus, these data essentially support those obtained in the maintenance

study and suggest that the assay is potentially useful to predict and support the morphological effects of pollutants on polyps. The technique may have use for studying other small macro-organisms. Preliminary studies also established mercury at 5×10^{-3}M to be inhibitory to polyp metabolism whereas arsenic is probably not inhibitory at 5×10^{-3}M for at least 20 hours of incubation.

On the basis of these studies, it is suggested that this combination of metabolic and maintenance studies offers a new means of determining and understanding the complex interactions of the sea nettle and its environment. With the system established, it is thus recommended that further studies with other pollutants and variables be conducted. Such studies could make additional significant contributions to an understanding of possible factors contributing to the sea nettle blooms in the Chesapeake Bay.

In our remote sensing studies, we sought a method whereby sea nettle medusae could be detected. Photographic studies comparing color ectachrome, GAF two-layer color film, and infrared false color film indicated that the best photographic contrast could be obtained with the latter film type. A Poloroid filter significantly increased the depth of visibility. Spectral studies, however, revealed no significant features in the jellyfish reflectance signature which could

be distinguished from water. Only by lyophilizing several jellyfish and producing a concentrated jellyfish powder could some small spectral signatures features be resolved. If this signature could be sharply enhanced, jellyfish relatively close to the surface could be detected. Extrapolation to total populations might then be made.

The most promising direct means of detecting jellyfish was by laser beam reflectance, perhaps combined with image intensification systems. For such a system, a red laser offers maximum advantages. Water turbidity would not pose serious limitations in the Chesapeake Bay except in the depths of the main channel. However, since surface reflection interferes, it is suggested that either: 1) the laser energy be transmitted in pulses to the underwater target and that the reflection sensor be turned on only to receive light reflected from beneath the surface, or, 2) that the laser detection system operate beneath the water surface.

As an indirect means of detecting jellyfish, the possibility exists that algae, by association with jellyfish, could provide a "jellyfish index." Our studies showed that algae are readily detected by color infrared photography although the number of cells required for detection is quite high (approximately 10^7 cells/ml). However, since algae also have

characteristic reflectance spectra, it may be possible to enhance the limits of detection by measuring ratios of reflectance at different wavelengths. Such a method could also prove valuable for distinguishing algal types (green versus blue-green algae).

In further development of a remote sensing scheme for sea nettle detection, then, it is recommended that efforts be concentrated on direct methods involving detection by laser reflectance or indirect methods related to algal detection.

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I. INTRODUCTION

The Chesapeake Bay is plagued with large numbers of sea nettles (Chrysaora quinquecirrha) which are present from spring until late summer. It is not known whether or not the sea nettle population has increased over the years since no efficient means of census taking has yet been devised. The effects of current levels of Bay pollutants on the species are also unknown. In view of the large recreational and economical impact of sea nettles, we have, in our program, sought a method for taking censuses of jellyfish by remote sensing, and have conducted an examination of the effects of pollutants on the sea nettle, Chrysaora quinquecirrha, the predominant species in the Chesapeake Bay. This report presents the findings of our nine-month investigation.

II. BACKGROUND

Due to pioneering efforts at the Chesapeake Bay Biological Laboratories at Solomons, Maryland, considerable information is available regarding the geographical and seasonal distribution, temperature and salinity tolerances, life cycle, toxin, and feeding requirements of Chrysaora quinquecirrha (1-4). In addition, an extensive bioliography of all literature concerning sea nettles has been prepared by the Virginia Institute of Marine Science (5).

Although sea nettles are found along the entire East Coast, the greatest density occurs in the Chesapeake Bay, especially in the upper waters of the Bay and its estuaries. This distribution may be governed by a preference for sheltered waters of low salinity rather than for the high salinity waters of the lower Bay and the open ocean (1, 3). Sea nettle medusae are prevalent in the summer, the density increasing with increasing water temperatures until the maximum density is reached in August and September (1). Two other large jellyfish, Cyanae and Aurelia, are also found in the Chesapeake Bay, but these are prevalent only in the winter months; Chrysaora is the dominant summer species.

The life cycle of Chrysaora quinquecirrha (1, 4) shows an alteration of generations (Figure 1). The free-swimming

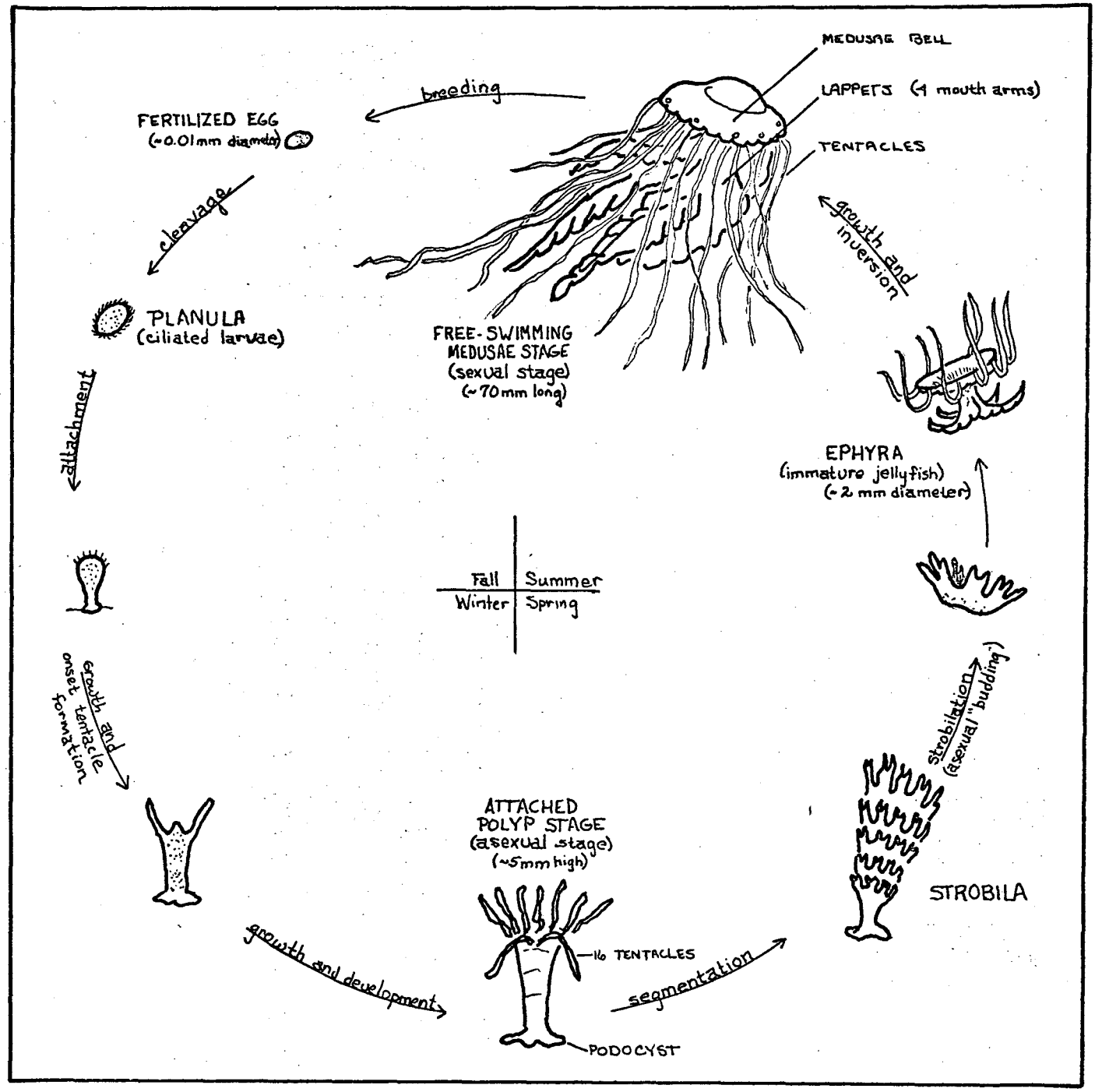

Figure 1

Life Cycle of the Sea Nettle Chrysaora quinquecirrha

The life cycle of Chrysaora quinquecirrha shows an alteration of generations between the free-swimming sexual medusae stage and the attached asexual polyp stage. Approximate seasonal relationships are indicated. Modified from Mansuetti (1).

medusa stage is the form familiar to summer swimmers and can reach diameters up to 12 inches with tentacles several feet in length. Adult medusae reproduce sexually in late summer to form a "planula" which attaches to the bottom of the Bay, elongates, and develops short tentacles. The resulting life stage, called a "polyp" is several millimeters in length and is the stage which survives the winter season. In the spring, polyps reproduce asexually by first forming segmented disks. The polyp then "buds" or "strobilates" when the terminal four or five disks separate from the main body of the polyp. These separated disks ("ephyra") are free-swimming, sexually differentiated, and gradually enlarge to become mature jellyfish medusae, thereby completing the life cycle.

The polyp is the sessile form of the jellyfish life cycle. In nature, polyps are distributed in relatively shallow bays or estuaries (1) in waters ranging from 7 - 20‰ in salinity. The importance of temperature and salinity in the regulation of polyp activities is readily demonstrated. Thus, strobilation can be induced either by raising the temperature above 20°C or by changing the salinity. It has been reported (2) that for polyps previously held at

15‰ salinity, strobilation was also induced either by lowering the salinity 5‰ or by raising it 5-10‰.

Polyps normally attach themselves to shells (especially oyster shells) or other bottom surfaces by means of a "cyst" or "podocyst." This brownish structure continually forms at the base of the polyp and varies in size and shape according to the length of time it has been forming. Upon removal of a podocyst from a polyp, the polyp attaches to a bottom surface and, after several weeks, a new podocyst mass becomes visible. Polyps also continually create new podocyst material by forming a "stolon" which is a budded structure on the posterior half of the body. This stolon gradually increases in size until it reaches and attaches to the surface adjacent to the existing attachment and eventually forms a new podocyst.

The continual formation of podocysts is apparently a means of increasing the polyp population since new polyps take form and grow directly on podocyst material. This polyp formation occurs even on podocyst material which has been isolated from all polyps. This latter method of asexual polyp reproduction appears to be the one used for the survival

of the species over the winter months when the water temperature is low (1). Thus, under such unfavorable conditions, polyps encyst and are no longer recognizable as polyps. When the temperature is again raised, new polyps gradually form on the podocyst material. Conditions reported (2) under which cyst formation has been observed are oxygen depletion concurrent with H_2S occurrence, a temperature decrease below $4^{\circ}C$, a temperature increase to 34° - $36^{\circ}C$, a salinity decrease below 5‰, or a salinity increase above 30‰.

Relatively little is known about the role of the sea nettle in the ecology and food chain of the Bay. According to Cargo and Schultz (2), the pelagic form of Chrysaora is preyed upon by harvest fish and the orange filefish whereas sea nettle planulae are food for plankton feeders. Nudibranch molluscs, or sea slugs, have been observed to devour polyps (3). Jellyfish are apparently carnivorous (6) and Chrysaora medusae may prey on Ctenophores (3).

III. MAINTENANCE OF CHRYSAORA QUINQUECIRRHA

1. Medusae Maintenance

During our precontract months and the first months of our contract period, sea nettle medusae were collected from the Chesapeake Bay and maintained in our laboratory at room temperature. The medusae selected were about two inches in diameter and could be maintained for at least two months in ten gallon acquaria with bottoms covered by oyster shells. Crowding was avoided and each tank contained approximately six individuals. The water of each tank was replaced twice weekly with fresh Bay water. It was also necessary to aerate the tanks, as has been reported for the maintenance of Aurelia aurita (7), and to change the aeration filters weekly.

Normal Bay water, as measured by $AgNO_3$ titration, contains approximately 12.7 g/l chloride which is approximately 20‰ salinity (8). Artificial sea water mixtures could not replace Bay water for supporting medusae. Two artificial mixtures were examined, one prepared from commercially available sea water salts and the other according to a formula (8) for artificial sea water. After preparation, each was equated with Bay water either by dilution to an appropriate chloride concentration by $AgNO_3$ titration or by dilution to

an appropriate density with the aid of a hydrometer. When matched to Bay water for density, both mixtures were low in chloride ion concentration but could support medusae for a day or two. Deviations from this density by 10%, however, resulted in death within a few hours. In view of these difficulties, it was concluded that Bay water was essential for successful maintenance of medusae over extended periods.

Medusae were fed tropical fish food daily. Occasionally, Ctenophores were caught from the Bay, cut into pieces, and also fed to the jellyfish. Both forms of food were ingested and could be seen within the digestive cavity of the medusae. In spite of this, medusae gradually underwent severe shrinkage over time until they finally reached an unrecognizable state. This shrinkage occurred regardless of initial size and was first noticed after about two weeks of maintenance. After about eight weeks, the medusae were reduced to a diameter of approximately 0.75 cm and after about ten weeks they were no longer recognizable. Similarly, Spangenberg (9) has reported that medusae of Aurelia aurita undergo "spontaneous deterioration" with time in captivity. Personnel at the Virginia Institute of Marine Science are also unable to maintain

Chrysaora medusae longer than this period and feel that starvation may account for the severe shrinkage (1) and that it probably also occurs in the Bay.

Since sea nettles disappear from the Bay in early fall, the last collection was made on 13 November 1970 and the medusae maintained as long as possible. During the winter months, Cyanae medusae became prevalent in the Bay. To continue our studies, several Cyanae medusae were received from Dr. Webb and Miss Olmon at the Virginia Institute of Marine Science and were maintained for several weeks at 4°C in a manner similar to that described above for Chrysaora. Although we had planned to conduct additional studies on Chrysaora medusae when they were anticipated in June, 1971, the medusae were not readily available from the Bay, even as late as the end of July. In fact, the summer of 1971 has witnessed a drastic reduction in the number of sea nettles present in the Bay, as illustrated in an excerpt from the Washington Post dated 7 August 1971 (Figure 2).

2. Polyp Maintenance

A stock collection of several hundred Chrysaora quinquecirrha polyps was donated for our studies by the Virginia Institute of Marine Science in November, 1970. Miss Janet Olmon, a graduate student at VIMS, described to

Less Sting in Swims As Jellyfish Decline

By Douglas Watson
Washington Post Staff Writer

The jellyfish that appear each summer in Chesapeake Bay and its estuaries, to the distress of bathers, are there in far fewer numbers this year.

Marine scientists aren't sure why, but they estimate there are only 10 to 15 per cent as many of the stinging sea nettles in the bay this summer as last year—itself a good year compared with the peak 1969 jellyfish season.

The chrysaora quinquecirrha, as they are known to scientists, are the target of the Federal Jellyfish Act of 1966, which authorized spending more than $1 million to find a way to eliminate the sea nettles from coastal waters, or at least neutralize their sting.

Two centers of this research effort are the Chesapeake Biological Laboratory at Solomons, Md., and the Virginia Institute of Marine Science in Gloucester, Va. Scientists at the two facilities said yesterday that this summer nature has, somehow, brought about the jellyfish reduction still being sought by researchers.

Dr. Dale R. Calder of the Virginia Institute said the great decrease in the millions of jellyfish that usually are floating in the bay at this time of year may be caused by unusually low oxygen levels in the deep water of the lower bay, a condition that threatens more desirable marine life as well.

However, Dr. David Cargo of the Chesapeake Biological Laboratory said that lack of oxygen in Virginia waters hasn't occurred in Maryland's portion of the bay and doesn't adequately explain the drop in jellyfish there.

"We had expected that we would have a lot of them this year," based on their sprawning, Dr. Cargo said.

However at the Solomons sampling site, where as many as 900 jellyfish were often collected during a typical five-day period in past summers, this year's five-day count has been as few as five or six. The nettles have been reported in the bay since colonial times.

Early August is usually the peak period for jellyfish, but because of weather conditions, this year's population is expected to increase slightly during this month. Dr. Calder warned that the jellyfish could be back next year in more typical numbers and "as venomous as ever."

The Virginia Institute is trying to develop a means to eradicate the creatures during their harmless polyp stage, which can last for years before the tiny hydra-like polyps that live on the bay bottom multiply into jellyfish, which survive for only a summer.

The Chesapeake Biological Laboratory is seeking chemical control agents, analyzing the nettles' stinging cells, seeking a natural predator that might reduce the jellyfish population and designing barriers to protect bathers.

It is also doing research on a possible injection that people could take before going swimming that would neutralize the jellyfish's sting—something that might be more popular on a beach trip than suntan lotion.

Figure 2

Excerpt from Washington Post of August 7, 1971 Regarding Declining Sea Nettle Population in the Chesapeake Bay

us in detail the morphological features of polyps, techniques for maintaining them, and techniques for inducing reproduction.

Our stock polyp collection was maintained in finger bowls at 20°C in a 20‰ artificial sea water mixture prepared according to Miss Olmon (Table 1). Once a week, polyps were removed from their finger bowls and placed in clean bowls containing fresh 20‰ artificial sea water. Polyps were fed live Artemia twice weekly. These brine shrimp were hatched in a covered vessel containing 20‰ artificial sea water (Table 1) at 80°F. The vessel was equipped with a screen net separating the covered portion of the vessel from a section exposed to light. The newly hatched shrimp are attracted to light and swim through the net. Here, they were easily collected by eye dropper and added to the polyp finger bowls. It was observed that feeding at intervals more frequent than twice weekly tended to dirty the water quickly and the polyp population size and growth were not significantly enhanced. Feeding at less frequent intervals was not detrimental to the polyp but did not allow the smaller polyps to grow at their optimal rate.

With adequate food, polyp numbers will increase largely through formation of new polyps on podocysts. However, this

Table 1

Polyp Medium [1]

SOLUTION A - Dissolve the following in approximately 2 liters of distilled water:

H_3BO_3	0.38 g
KBr	1.39 g
$NaHCO_3$	2.79 g
KCl	9.64 g
Na_2SO_4	56.8 g
$MgCl_2 \cdot 6H_2O$	154.4 g
NaCl	340.7 g

SOLUTION B - Dissolve 21.29 g of $CaCl_2$ in a small amount of distilled water.

SOLUTION C - Dissolve 0.10 g of KI in 100 ml of water.

Add distilled water to Solution A to bring the volume to 10 liters. Add Solution B and 1.0 ml of Solution C in with the last liter. The pH of the final Solution should be appromixately 8.0. This stock solution (50‰ artificial sea water) must be diluted to 20‰ for use as the polyp medium.

1/. As obtained from Miss Janet Olmon from the Virginia Institute of Marine Science.

rate of increase was too slow to develop the large number of polyps required for our program. Consequently, we studied faster methods of increasing our stock polyp supply. Two methods were examined:

1. Podocysts were removed from each of our 300 polyps. The detached polyps were allowed to recover for a few days before subjecting them to additional stresses of other experiments whereas the podocysts were placed at 4°C. After four weeks at this low temperature, these podocysts were expected to develop into new polyps upon raising the temperature to about 20°C. However, while new polyps did develop under these conditions, the recovery rate was only about 10-20%. The low recovery rate and the long time period required make this method unsatisfactory for increasing polyp numbers.

2. Both portions of a severed polyp are expected to regenerate into complete polyps. Consequently, several polyps (without podocysts) were cut to determine the optimal sectioning (Figure 3). Cuts were made longitudinally and in cross-

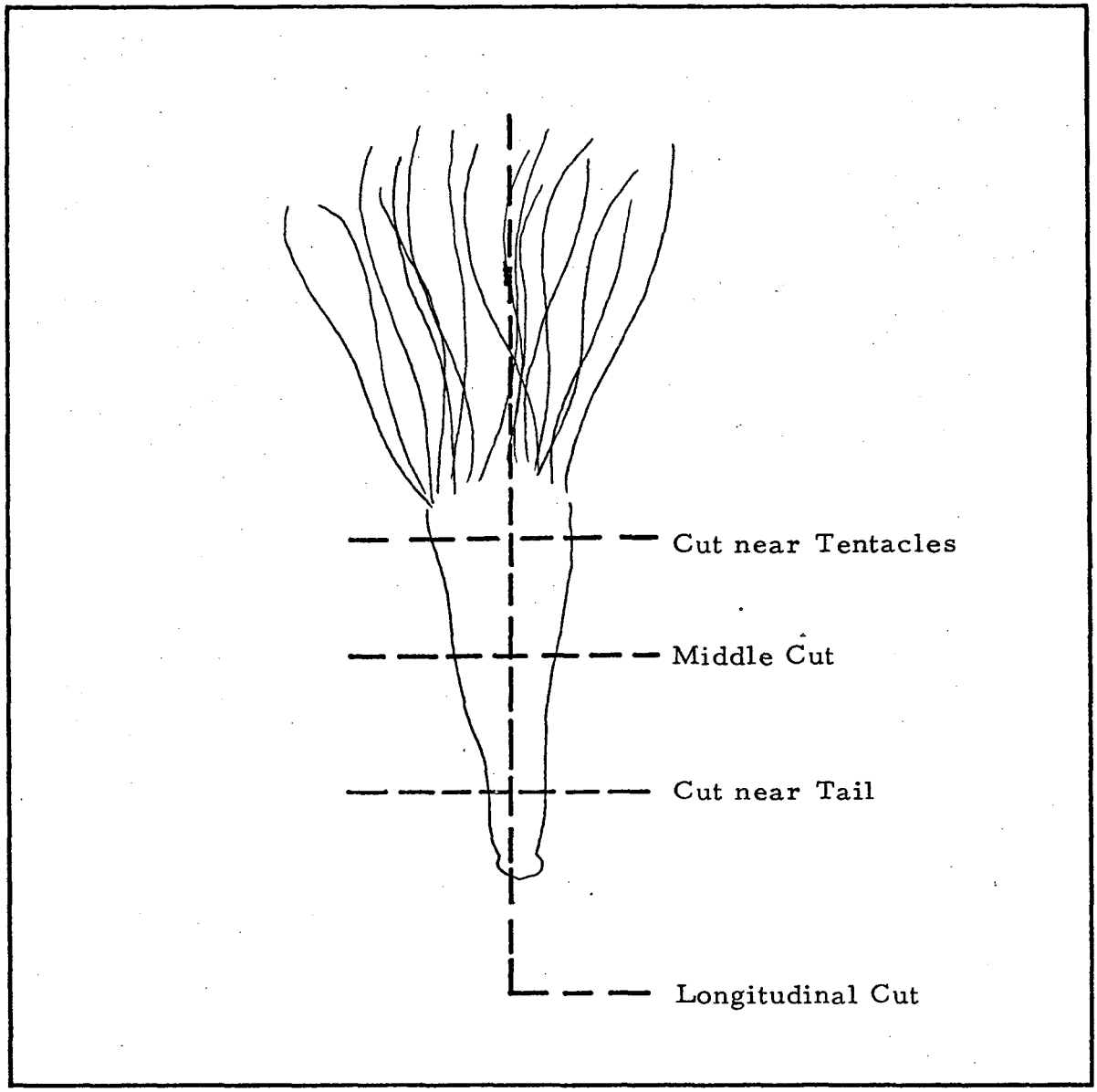

Figure 3

Sketch of Sea Nettle Polyp Indicating Approximate Levels of Sectioning in Regeneration Experiment

At each indicated level, 10 polyps were sectioned and the resulting halves observed for successful regeneration over the following weeks. Results are presented in Table II.

section near the tentacles, in the middle, and near the tail of the polyp. Ten polyps were severed at each level and the sections maintained at 26°C for three or four weeks. During this time, the sections were observed for survival and time to regenerate a normal appearance.

The results (Table 2) indicate that for longitudinal sectioning, 90% of the sections survive and appear normal in size after about two weeks. However, many of the resulting polyps were twisted in shape showing, for example, a pronounced bend in the middle of the body. For cross-section cuts, 100% of the tentacle sections recover regardless of the position of the cut. This may reflect the ability of these sections to continue eating brine shrimp after sectioning. However, the closer the cut to the tentacles, the longer the time required for the tentacle section to appear as a "normal" polyp. Approximately two to three weeks are required for full recovery from a cut made close to the tentacles. Tail sections obtained from a cut close to the tentacle show 90% survival and require four to five weeks to regenerate tentacles and appear normal. Tail sections from cuts further removed from the tentacles show a correspondingly longer time to regenerate and a smaller survival rate.

Table 2

Percent Survival and Recovery Time for Regeneration
Following Polyp Sectioning at Various Levels

Type Section	Level of Cut							
	Cross Section Near Tentacles		Cross Section Near Middle		Cross Section Near Tail		Longitudinal Section	
	Recovery Time	Percent	Recovery Time	Percent	Recovery Time	Percent	Recovery Time	Percent
Top Halves	Two Weeks	100	Three Weeks	100	One Week	100		
Bottom Halves	Four Weeks	90	Four Weeks	75	Seven Weeks	50		
Longitudinal Halves							Four Weeks	90

Four groups of ten polyps each were severed at the indicated level (see Figure 3 for position of cut). The sections were then separated (except longitudinal sections), maintained in 20‰ artificial sea water, and observed for survival and time to regenerate a "normal" appearance.

On the basis of these data, it may be concluded that the quickest and most reliable means of increasing the polyp population is to sever polyps in cross-section at a level near the tentacles. Consequently, our stock supply of polyps was increased and routinely maintained throughout this program by cutting 50 polyps in cross-section close to the tentacles every two weeks.

3. Selection of Life Form for Experimental Studies

The remote sensing aspect of this program is aimed at medusae detection in the Chesapeake Bay and, consequently, requires utilization of the medusae stage of Chrysaora quinquecirrha. However, for the pollution aspects of this program, medusae are undesirable since they are relatively large and difficult to maintain. To conduct statistically valid, reproducible studies, large numbers of individuals are required. Since polyps are small of size and can be easily maintained over extended periods, they are the life stage of choice for examining the effects of pollutants on Chrysaora. Further, it seems likely that any effective measure to control the medusae population must be aimed at the polyp stage which is the source.

IV. POLLUTION STUDIES

A. Polyp Maintenance

1. Effect of Pollutants on Maintenance of Chrysaora quinquecirrha Polyps

To determine the effects of pollutants on polyps, polyps were maintained in 20‰ artificial sea water (Table 1) to which had been added varying levels of several different pollutants. The pollutants under examination were phosphate, nitrate, ammonium, a combination of these three pollutants, and synthetic sewage effluent. The selection of pollutants and their concentration range was made on the basis of known occurrence in typical polluted waters. The specific concentrations examined are as follows:

Phosphate: 0.05, 0.25, 1.0, 5.0, and 20.0 ppm as phosphorus

Nitrate: 1, 3, 10, 30, and 100 ppm as nitrogen

Ammonium: 1, 3, 10, 30, and 100 ppm as nitrogen

Five Combinations of the Above Three Pollutants:

0.05 ppm phosphate-P + 1 ppm nitrate-N + 1 ppm ammonium-N
0.25 ppm phosphate-P + 3 ppm nitrate-N + 3 ppm ammonium-N
1.0 ppm phosphate-P + 10 ppm nitrate-N + 10 ppm ammonium-N
5.0 ppm phosphate-P + 30 ppm nitrate-N + 30 ppm ammonium-N
20.0 ppm phosphate-P + 100 ppm nitrate-N + 100 ppm ammonium-N

Synthetic Sewage Effluent: 1, 3, 10, 20, 50% (by volume; however, media were prepared such that the final concentration of the artificial sea water remained 20‰). Synthetic sewage effluent contains a combination of phosphate, nitrate, ammonium, and, in addition, organic material.

The pH of each test environment was maintained at 7.8 which is a value close to that of natural Bay water. However, since many of these pollutants could lower the pH of the medium, an additional set of test environments was examined in which only the pH of the 20‰ artificial sea water was varied as follows:

pH Values: 6.2, 6.8, 7.0, 7.4

Four polyps were initially placed in 25 ml of each of the above 29 test environments and compared to three control environments each of which contained four polyps in 20‰ artificial sea water at pH 7.8.

Control and test environments containing the indicated levels of the selected pollutants have also been incubated with polyps at each of three different temperatures (5°, 20°, and 26° C). In the early stages of our study, this experimental design allowed determination of the temperature dependence of any pollutant effect. Further, it allowed a study of the effect of pollutants on those polyp activities which are temperature dependent. Thus, at 4° (a winter temperature),

polyps are known to encyst. Sharp temperature increases after long-term storage at either 4^{o} or 20^{o} also affect polyp activities. Polyps maintained for 12 weeks at 20^{o} are expected to strobilate upon raising the temperature to 26^{o}. After long-term maintenance at 4^{o}, a temperature increase is expected to stimulate cysts to form polyps. Thus, in the latter stages of our study, increasing the incubation temperature could allow examination of the effects of pollutants on strobilation and on winter survival of polyps. Consequently, the following incubation conditions were compared for each control and test environment:

1. Polyps were incubated at 26^{o}C for the 21 week duration of the experiment.

2. Polyps were incubated at 20^{o}C for 13 weeks and then raised to 26^{o} for eight additional weeks.

3. Polyps were incubated at 5^{o}C for ten weeks, raised to 20^{o} for three weeks, and then raised to 26^{o} for the remaining eight weeks of the experiment.

Routine maintenance of this experiment involved daily feeding of all the polyps with brine shrimp and weekly replacement of each of the 96 test and control media (32 at each temperature). All polyps were carefully monitored for morphological changes that occurred with time. Initially,

daily records were kept, but, as the experiment progressed, only weekly records were necessary.

In monitoring this experiment for the effects of pollutants, the most important measure was a count of the number of viable polyps present in each environment and their state of health. Observation established that normal healthy polyps catch and eat brine shrimp, have slender bodies slightly pinkish in color, and have relaxed tentacles about the length of the body itself. Thus, a polyp is considered healthy if it satisfies these requirements of feeding, body shape, color, and tentacle length. Moving toward death, a polyp first contracts its tentacles, then quickly loses color and turns pale. Finally, the body contracts, shrinking into a ball-like shape. It is not clear, however, what actually constitutes death since in this experiment the shrunken, contracted, pale polyp could not be moved into a more favorable environment to test for recovery.

The weekly polyp counts for each control and test environment are presented in Tables 3 through 9, Table 3 shows the individual and average results obtained from controls lacking added pollutants. The other tables separate each pollutant and present polyp numbers as a function of contact

TEMP	Control Number	VIABLE POLYP COUNT AT END OF INDICATED WEEK																				
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
26°	1	4	5	5	5	6	-	5	5	5	5	6	6	5	6	6	6	-	-	7	7	7	6
	2	4	4	5	5	5	-	7	6	7	8	9	10	10	11	11	11	-	-	12	12	12	11
	3.	4	5	5	5	5	-	5	6	7	9	10	8	9	9	11	12	-	-	12	14	13	14
	Average	4.0	4.7	5.0	5.0	5.3	-	5.7	5.7	6.3	7.3	8.3	8.0	8.0	8.7	9.3	9.7	-	-	10.3	11.0	10.7	10.3
20°	1	4	5	5	5	5	-	5	5	6	6	6	6	6	6 raise temperature to 26°C	6	6	-	-	6	6	6	6
	2	4	4	4	4	4	-	4	4	4	4	4	4	4	4 raise temperature to 26°C	4	4	-	-	5	5	5	6
	3	4	4	3	4	4	-	3	4	4	5	4	5	5	5 raise temperature to 26°C	5	5	-	-	4	5	5	5
	Average	4.0	4.3	4.0	4.3	4.3	-	4.0	4.3	4.7	5.0	4.7	5.0	5.0	5.0 raise temperature to 26°C	5.0	5.0	-	-	5.0	5.3	5.3	5.3
5	1	4	E	E	E	E	-	E	E	E	E	E raise temperature to 20°C	0	0	0 raise temperature to 26°C	0	0	-	-	0	0	0	0
	2	4	E	E	E	E	-	E	E	E	E	E	0	0	0	1	2	-	-	1	1	2	1
	3	4	E	E	E	E	-	E	E	E	E	E	0	0	0	3	3	-	-	3	3	3	3
	Average	4	E	E	E	E	-	E	E	E	E	E	0	0	0	1.3	1.7	-	-	1.3	1.3	1.7	1.3

Table 3

Polyp Survival and Multiplicity in 20‰ Artificial Sea Water at Three Different Temperatures

At each of the three indicated temperatures, 4 polyps were placed into each of three control environments containing approximately 25 ml of 20‰ artificial sea water at pH 7.8. Fresh media were supplied weekly and polyps were fed twice weekly with brine shrimp. Polyps were observed for morphological changes and counted once a week. The letter "E" is used to indicate that all polyps shrank and encysted. Temperature raises after 10 and 13 weeks are also indicated.

TEMP	pH	VIABLE POLYP COUNT AT END OF INDICATED WEEK																					
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
26°	7.4	4	4	4	4	4	-	5	5	5	5	5	5	5	5	6	6	-	-	8	8	7	8
	7.0	4	4	4	4	5	-	6	6	5	6	6.	6	6	5	5	6	-	-	5	5	5	5
	6.8	4	4	4	5	4	-	6	6	6	6	6	9	8	12	12	15	-	-	15	14	14	15
	6.2	4	4	4	4	4	-	4	5	5	5	6	7	8	8	8	8	-	-	9	9	9	9
20°	7.4	4	4	4	4	4	-	4	4	4	4	4	4	4	4 raise temperature to 26°C	5	5	-	-	6	6	6	8
	7.0	4	4	4	4	4	-	5	4	4	4	4	4	4	4 raise temperature to 26°C	4	6	-	-	10	8	8	9
	6.8	4	4	4	4	4	-	4	4	4	4	4	4	4	4 raise temperature to 26°C	4	4	-	-	4	4	5	4
	6.2	4	4	5	5	5	-	5	5	5	5	4	4	4	4 raise temperature to 26°C	4	4	-	-	4	4	4	4
5°	7.4	4	E	E	E	E	-	E	E	E	E	E raise temperature to 20°C	0	0	0 raise temperature to 26°C	0	0	-	-	0	0	0	0
	7.0	4	E	E	E	E	-	E	E	E	E	E raise temperature to 20°C	0	0	0 raise temperature to 26°C	1	3	-	-	3	3	3	3
	6.8	4	E	E	E	E	-	E	E	E	E	E raise temperature to 20°C	0	0	0 raise temperature to 26°C	1	1	-	-	1	1	1	1
	6.2	4	E	E	E	E	-	E	E	E	E	E raise temperature to 20°C	0	0	0 raise temperature to 26°C	0	0	-	-	0	0	0	0

Table 4

Effect of pH on Polyp Survival and Multiplicity in 20‰ Artificial Sea Water at Three Different Temperatures

At each of the three indicated temperatures, 4 polyps were placed into each of four test environments containing approximately 25 ml of 20‰ artificial sea water adjusted to the indicated pH. Maintenance conditions are as described for Table 3.

TEMP	ppm-P	VIABLE POLYP COUNT AT END OF INDICATED WEEK																				
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
26°	0.05	4	9	18	15	16	-	16	19	19	21	23	24	23	23	26	29	-	-	29	29	30	30
	0.25	4	4	4	6	6	-	7	7	7	7	7	7	7	8	7	8	-	-	9	9	9	9
	1.0	4	6	6	6	6	-	8	8	8	8	8	8	8	8	8	7	-	-	7	8	7	8
	5.0	4	7	7	7	8	-	9	10	9	12	12	13	13	13	13	14	-	-	15	15	14	14
	20.0	4	4	4	5	4	-	4	4	5	5	5	5	7	7	6	7	-	-	7	6	7	7
20°	0.05	4	4	5	4	5	-	6	6	6	6	6	6	6	6 raise temperature to 26°C	6	6	-	-	6	6	4	5
	0.25	4	4	5	5	5	-	5	4	4	6	5	5	5	6 raise temperature to 26°C	5	7	-	-	9	10	10	11
	1.0	4	4	4	4	4	-	5	4	4	6	5	6	5	6 raise temperature to 26°C	6	6	-	-	6	6	6	6
	5.0	4	4	3	3	6	-	6	6	5	5	6	6	6	6 raise temperature to 26°C	6	6	-	-	7	6	7	7
	20.0	4	4	4	4	4	-	4	4	4	6	4	5	4	4 raise temperature to 26°C	4	4	-	-	5	4	4	4
5°	0.05	4	E	E	E	E	-	E	E	E	E	E raise temperature to 20°C	0	0	0 raise temperature to 26°C	0	0	-	-	2	2	2	2
	0.25	4	E	E	E	E	-	E	E	E	E	E raise temperature to 20°C	0	0	0 raise temperature to 26°C	1	2	-	-	3	3	3	2
	1.0	4	E	E	E	E	-	E	E	E	E	E raise temperature to 20°C	0	1	1 raise temperature to 26°C	1	1	-	-	1	1	1	1
	5.0	4	E	E	E	E	-	E	E	E	E	E raise temperature to 20°C	0	0	0 raise temperature to 26°C	0	0	-	-	0	0	0	0
	20.0	4	E	E	E	E	-	E	E	E	E	E raise temperature to 20°C	0	0	0 raise temperature to 26°C	0	0	-	-	0	0	0	0

Table 5

Effect of Phosphate on Polyp Survival and Multiplicity at Three Different Temperatures

At each of the three indicated temperatures, 4 polyps were placed into each of five test environments containing approximately 25 ml of 20‰ artificial sea water to which had been added varying amounts of phosphate (as ppm phosphorus), as indicated. The pH of each medium was maintained at 7.8. Maintenance conditions are as described for Table 3.

TEMP	ppm-N	VIABLE POLYP COUNT AT END OF INDICATED WEEK																					
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
26°	1	4	4	4	5	5	-	5	6	6	5	7	8	7	8	8	8	-	-	9	9	8	8
	3	4	5	5	4	5	-	5	5	5	6	7	7	9	9	8	9	-	-	11	10	11	11
	10	4	5	6	7	8	-	11	12	12	12	14	13	16	15	18	18	-	-	21	21	20	21
	30	4	4	4	4	4	-	4	4	4	6	7	9	9	10	10	13	-	-	12	12	13	12
	100	4	4	6	6	7	-	7	7	7	7	7	7	7	7	7	7	-	-	5	6	6	6
20°	1	4	4	4	5	4	-	5	5	5	5	5	5	5	6	5	6	-	-	5	5	5	5
	3	4	5	5	5	4	-	5	5	5	6	6	6	6	6	6	6	-	-	6	6	6	6
	10	4	5	5	5	6	-	6	6	6	6	6	6	6	6	5	5	-	-	9	9	7	11
	30	4	4	3	2	2	-	2	2	2	2	2	2	2	2	2	2	-	-	4	3	3	4
	100	4	4	5	5	5	-	4	4	4	4	5	5	5	5	5	5	-	-	5	5	4	5
5°	1	4	E	E	E	E	-	E	E	E	E	E	0	0	0	1	2	-	-	1	1	1	1
	3	4	E	E	E	E	-	E	E	E	E	E	0	0	0	1	2	-	-	2	2	1	2
	10	4	E	E	E	E	-	E	E	E	E	E	0	0	0	1	1	-	-	1	1	1	1
	30	4	E	E	E	E	-	E	E	E	E	E	0	0	0	1	1	-	-	1	1	1	1
	100	4	E	E	E	E	-	E	E	E	E	E	0	0	0	0	0	-	-	0	0	0	0

raise temperature to 20°C

raise temperature to 26°C

Table 6

Effect of Nitrate on Polyp Survival and Multiplicity at Three Different Temperatures

At each of the three indicated temperatures, 4 polyps were placed into each of five test environments containing approximately 25 ml of 20‰ artificial sea water to which had been added varying amounts of nitrate (as ppm nitrogen), as indicated. The pH of each medium was maintained at 7.8. Maintenance conditions are as described for Table 3.

TEMP	ppm-N	VIABLE POLYP COUNT AT END OF INDICATED WEEK																				
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
26°	1	4	4	4	4	4	-	4	6	6	6	8	9	5	5	7	8	-	-	8	8	5	8
	3	4	4	4	4	4	-	4	4	4	5	5	5	5	4	4	4	-	-	4	4	3	4
	10.	4	3	3	2P	2P	-	2P	2P	2P	1P	1P	1P	1P	1	1	1	-	-	1	1	1	0
	30	4	3P	2P	2P	4P	-	0	0	0	0	0	0	0	0	0	0	-	-	0	0	0	0
	100	4	2	2P	3P	3P	-	0	0	0	0	0	0	0	0	0	0	-	-	0	0	0	0
20°	1	4	4	4	4	4	-	4	4	4	4	4	4	4	raise temperature to 26°C	4	4	-	-	4	4	3	5
	3	4	4	4	4	4	-	4	4	4	4	4	4	4		4	4	-	-	4	4	4	4
	10	4	4	4	2	2	-	2P	2P	2P	1	1	1	2	2	2	2	-	-	2	2	2	2
	30	4	4P	3P	2P	2P	-	0	0	0	0	0	0	0	0	0	0	-	-	0	0	0	0
	100	4	4P	2P	2P	4P	-	0	0	0	0	0	0	0	0	0	0	-	-	0	0	0	0
5°	1	4	E	E	E	E	-	E	E	E	E	E	raise temperature to 20°C	0	0	raise temperature to 26°C	0	0	-	-	0	0	0	0
	3	4	E	E	E	E	-	E	E	E	E	E		0	0		1	1	-	-	1	1	1	1
	10	4	E	E	E	E	-	E	E	E	E	E		0	0		0	0	-	-	0	0	0	0
	30	4	E	E	E	E	-	E	E	E	E	E		0	0		0	0	-	-	0	0	0	0
	100	4	E	E	E	E	-	E	E	E	E	E		0	0		0	0	-	-	0	0	0	0

Table 7

Effect of Ammonium on Polyp Survival and Multiplicity at Three Different Temperatures

At each of the three indicated temperatures, 4 polyps were placed into each of five test environments containing approximately 25 ml of 20‰ artificial sea water to which had been added varying amounts of ammonium chloride (as ppm nitrogen), as indicated. The pH of each medium was maintained at 7.8. Maintenance conditions are as described for Table 3. The letter "E" indicates polyp encystment whereas "P" represents disintegration of polyps to "pieces".

TEMP	Comb #	VIABLE POLYP COUNT AT END OF INDICATED WEEK																				
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
26°	1	4	4	5	4	4	-	6	8	8	10	11	11	10	12	11	10	-	-	12	13	10	11
	2	4	4	5	4	5	-	5	5	5	6	7	10	9	9	8	12	-	-	9	12	11	10
	3	4	6	6	5	5	-	5	5	5	5	5	5	5	6	6	6	-	-	5	6	6	6
	4	4	4	4	4	4	-	3	2P	3P	0	0	0	0	0	0	0	-	-	0	0	0	0
	5	4	2P	2P	2P	2P	-	0	0	0	0	0	0	0	0	0	0	-	-	0	0	0	0
20°	1	4	3	4	4	4	-	3	3	3	3	3	3	3	3	3	4	-	-	4	4	4	4
	2	4	4	4	4	4	-	4	5	5	5	5	5	5	5	5	5	-	-	4	6	6	6
	3	4	4	4	4	4	-	4	4	4	4	4	4	4	4	4	4	-	-	4	3	3	3
	4	4	4	4	4	4P	-	4P	3P	0	0	0	0	0	0	1	1	-	-	0	0	0	0
	5	4	3P	3P	3P	3P	-	3P	0	0	0	0	0	0	0	0	0	-	-	0	0	0	0
5°	1	4	E	E	E	E	-	E	E	E	E	E	0	0	0	1	1	-	-	1	1	1	1
	2	4	E	E	E	E	-	E	E	E	E	E	0	0	0	0	0	-	-	0	0	0	0
	3	4	E	E	E	E	-	E	E	E	E	E	0	0	0	0	0	-	-	0	0	0	0
	4	4	E	E	E	E	-	E	E	E	E	E	0	0	0	0	0	-	-	0	0	0	0
	5	4	E	E	E	E	-	E	E	E	E	E	0	0	0	0	0	-	-	0	0	0	0

raise temperature to 20°C

raise temperature to 26°C

raise temperature to 26°C

Table 8

Effect of Combinations of Phosphate, Nitrate, and Ammonium on Polyp Survival and Multiplicity

At each of the three indicated temperatures, 4 polyps were placed into each of five test environments containing approximately 25 ml of 20‰ artificial sea water to which had been added various combinations of phosphate, nitrate, and ammonium (Combination #1 = 0.05 ppm-PO_4-P + 1 ppm-NO_3-N + 1 ppm-NH_4-N; Comb #2 = 0.25 ppm-PO_4-P + 3 ppm-NO_3-N + 3 ppm-NH_4-N; Comb #3 = 1 ppm-PO_4-P + 10 ppm-NO_3-N + 10 ppm-NH_4-N; Comb #4 = 5 ppm-PO_4-P + 30 ppm-NO_3-N + 30 ppm-NH_4-N; Comb #5 = 20 ppm-PO_4-P + 100 ppm-NO_3-N + 100 ppm-NH_4-N). The pH of each medium was maintained at 7.8. Maintenance conditions are described

TEMP	%	VIABLE POLYP COUNT AT END OF INDICATED WEEK																				
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
26°	1	4	4	4	5	4	-	5	5	5	8	8	9	10	10	11	10	-	-	12	10	12	10
	3	4	4	4	4	4	-	4	4	4	4	4	4	4	4	4	4	-	-	4	4	4	4
	10	4	4	4	5	4	-	5	5	4	5	4	4	5	4	4	4	-	-	3	4	4	4
	20	4	4	4P	2P	3P	-	3P	0	0	0	0	0	0	0	0	0	-	-	0	0	0	0
	50	4	4	4P	4P	2P	-	3P	0	0	0	0	0	0	0	0	0	-	-	0	0	0	0
20°	1	4	4	4	4	4	-	4	4	4	4	4	4	4	4	4	4	-	-	4	4	4	5
	3	4	4	4	4	4	-	4	5	5	5	5	5	5	5	5	5	-	-	9	8	8	7
	10	4	4	4	4	4	-	4	4	5	4	4	4	4	4	4	4	-	-	5	5	6	6
	20	4	4P	4P	2P	2P	-	2P	2P	0	0	1	1	2	2	2	2	-	-	2	2	2	2
	50	4	4P	4P	2P	1P	-	3P	0	0	0	0	0	0	0	0	0	-	-	0	0	0	0
5°	1	4	E	E	E	E	-	E	E	E	E	E	0	0	0	0	1	-	-	1	1	1	1
	3	4	E	E	E	E	-	E	E	E	E	E	0	0	0	0	0	-	-	1	2	2	3
	10	4	E	E	E	E	-	E	E	E	E	E	0	0	0	0	0	-	-	0	0	0	0
	20	4	E	E	E	E	-	E	E	E	E	E	0	0	0	0	0	-	-	0	0	0	0
	50	4	E	E	E	E	-	E	E	E	E	E	0	0	0	0	0	-	-	0	0	0	0

raise temperature to 20°C

raise temperature to 26°C

raise temperature to 26°C

Table 9

Effect of Synthetic Sewage Effluent on Polyp Survival and Multiplicity at Three Different Temperatures

At each of the three indicated temperatures, 4 polyps were placed into each of five test environments containing the indicated percentage of synthetic sewage effluent in 20‰ artificial sea water. The pH of each medium was maintained at 7.8. Maintenance conditions are described under Table III and the letter code is given in Table 7.

time for each pollutant concentration at each temperature. The difficulties described above in determining time of death and in distinguishing live polyps from dead pieces somewhat complicate polyp tallies and, no doubt, introduced small fluctuations in the weekly counts recorded for each environment. However, in spite of these fluctuations, polyp counts readily reveal trends resulting from each environmental condition.

Although Tables 3 through 9 are conveniently separated according to pollutant, it is more convenient to discuss the results according to the temperature of incubation. The following is a summary of the results obtained from maintaining polyps under various conditions at constant temperature.

a). At 26°C, a summer temperature, all polyps were maintained for a total of 21 weeks. During this period, control polyps (Table 3) appear healthy and gradually increase in numbers. However, considerable variation in percent increase was observed among each of the three replicate control environments. Both the average increase and the observed range, shown in Table 3, serve as standards of comparison for the test environments. Essentially no differences from these controls are obtained by lowering the

pH of the environment from 7.8 to 6.2 (Table 9).

In the presence of added phosphate (Table 5) or nitrate (Table 6), all polyps appear healthy throughout the concentration ranges examined and are increasing in number as are the control polyps. However, at one phosphate concentration (0.25 ppm phosphate phosphorus), the polyps increase in number from four to 30, an increase significantly above the four to ten average increase seen in the controls. Similarly, at one nitrate concentration (10 ppm nitrogen), the polyp number increases from four to 21, an increase also well above that observed for controls. At all other phosphate or nitrate concentrations, the observed increase is within range of the controls, although in some cases it is above the average increase in the controls.

Added ammonium has a detrimental effect on polyps (Table 7). At the lowest concentration examined (1 ppm ammonium nitrogen), polyps appear normal, but their rate of increase is below that of the controls. Increasing the concentration to 3 ppm prevents any increase in polyp numbers from occurring. At 10 ppm, polyps contract and become unhealthy in appearance. Gradually, these

polyps disintegrate with time. Similar, but more severe, effects are observed within one week of exposure to higher concentrations of ammonium.

Results obtained with added combinations of ammonium + nitrate + phosphate (Table 8) are similar to those seen in the presence of ammonium alone although there is apparently a protective effect of nitrate and phosphate. For example, at 10 ppm ammonium alone, polyps disintegrate. At 10 ppm ammonium plus phosphate and nitrate, polyps appear normal, but do not increase in number (a result also obtained with 3 ppm ammonium nitrogen alone).

Synthetic sewage effluent (Table 9) is also detrimental to polyps in a manner similar to that observed for added ammonium. Thus, the test environment containing 1% synthetic sewage effluent supports healthy polyps which increase in number. Polyp multiplication is arrested in 3% and 10% synthetic sewage effluent whereas at higher concentrations polyps gradually disintegrate.

b). At $20^{\circ}C$, a spring temperature, polyps were maintained for only 13 weeks before raising the temperature. During these 13 weeks, results obtained were similar to those observed at 26° except that polyps do not multiply as

rapidly at the lower temperature. Thus, control polyps at 20° showed an average increase from four to five compared to an average increase from four to nine among controls at 26°. No effect of lower pH values was obtained. Polyps in all concentrations of phosphate and nitrate are healthy but do not significantly increase in number above control values. The detrimental effects of ammonium, combinations of ammonium + phosphate + nitrate, and synthetic sewage effluent are about as severe at 20° as at 26°.

c). At 5°C, a winter temperature, polyps were maintained for only ten weeks before raising the incubation temperature. Within one week of incubation at 5°, all polyps were reduced to $\frac{1}{4}$ or 1/8 of their original size, had severely shrunken tentacles, and appeared as undefined protoplasmic material. No differences among control and test environments were evident and no further changes were seen during the remaining nine weeks at this incubation temperature.

After maintaining all environments at each constant temperature for the specified time intervals, the temperature of those polyps initially incubated at either 5° or 20° was raised. No temperature changes were made to those polyps initially maintained at 26°C. The resulting changes in polyp

number are also shown in Tables 3 through 9 and may be summarized as follows:

Polyps maintained at 20° for 13 weeks and then raised to 26° for an additional eight weeks began to proliferate in the presence of added phosphate and nitrate. Most pronounced was the increase seen in the presence of 0.25 ppm and 5.0 ppm phosphate phosphorus and of 10 ppm nitrate nitrogen. These increases are comparable to those seen with polyps incubated only at 26° and occur at the same pollutant concentrations. Control polyps do not show corresponding increases although some polyps maintained at lower pH do exhibit such increases in numbers. Increasing the temperature does not cause a further detrimental effect of ammonium, combinations of ammonium plus phosphate plus nitrate, or synthetic sewage effluent.

Polyps maintained at 5° for ten weeks, raised to 20° for three weeks, then raised to 26° for eight additional weeks resembled protoplasmic masses after the 5° incubation. With the rise in temperature, small polyps became recognizable and slowly increased in size. In the presence of added phosphate or nitrate, the number of polyps formed did not differ significantly from the controls. These new polyps always

formed on podocyst material and no polyps per se survived the cold treatment. Approximately 10-20% of the podocysts gave rise to at least one new polyp. Added ammonium, sewage effluent, or pollutant combinations inhibited polyp formation at all concentrations except the lowest. No effect of the stepwise temperature increase was observed on polyp numbers.

Polyp strobilation normally follows a sharp temperature increase and the temperature raises in this experiment were designed to determine the effects of pollutants on strobilation. Our results confirm the necessity of a temperature rise for the onset of strobilation. Thus, polyps maintained at 26° for the entire duration of this experiment did not strobilate whereas many of the polyps which experienced a temperature increase did strobilate. Among the controls initially maintained at 20°, strobilation began within one week after raising the temperature to 26° and was essentially completed within about five weeks (although an occasional polyp was strobilating at the termination of the experiment). Among those polyps maintained first at 5°, then raised to 20° for three weeks before raising the temperature to 26°, strobilation occurred only in a small number of polyps, even among the pollution-free controls.

Contrary to expectation, strobilation did not occur synchronously within any given test environment, even among the pollution-free controls. For example, in an environment containing four healthy polyps, only one may strobilate (Figure 4) within eight weeks. This asynchrony occurs both with respect to the number of polyps strobilating and with respect to the time of onset of strobilation.

The effects of pollutants on strobilation are not completely clear. In all test situations which contained healthy polyps initially at 20°, usually at least one polyp strobilated. However, there were no clear examples of stimulation, inhibition, or of a concentration effect of a particular pollutant on strobilation. One difficulty in establishing clear patterns is the lack of synchrony within each environmental situation. However, for those polyps maintained first at 5°, strobilation occurred only among controls and among polyps maintained either in phosphate, nitrate, or low concentrations of ammonium.

In brief summary of this experiment, then, the effect of pollutants is most obvious at high temperatures and is more pronounced on polyp numbers than on polyp strobilation. Compared to pollution-free controls, polyp numbers have been

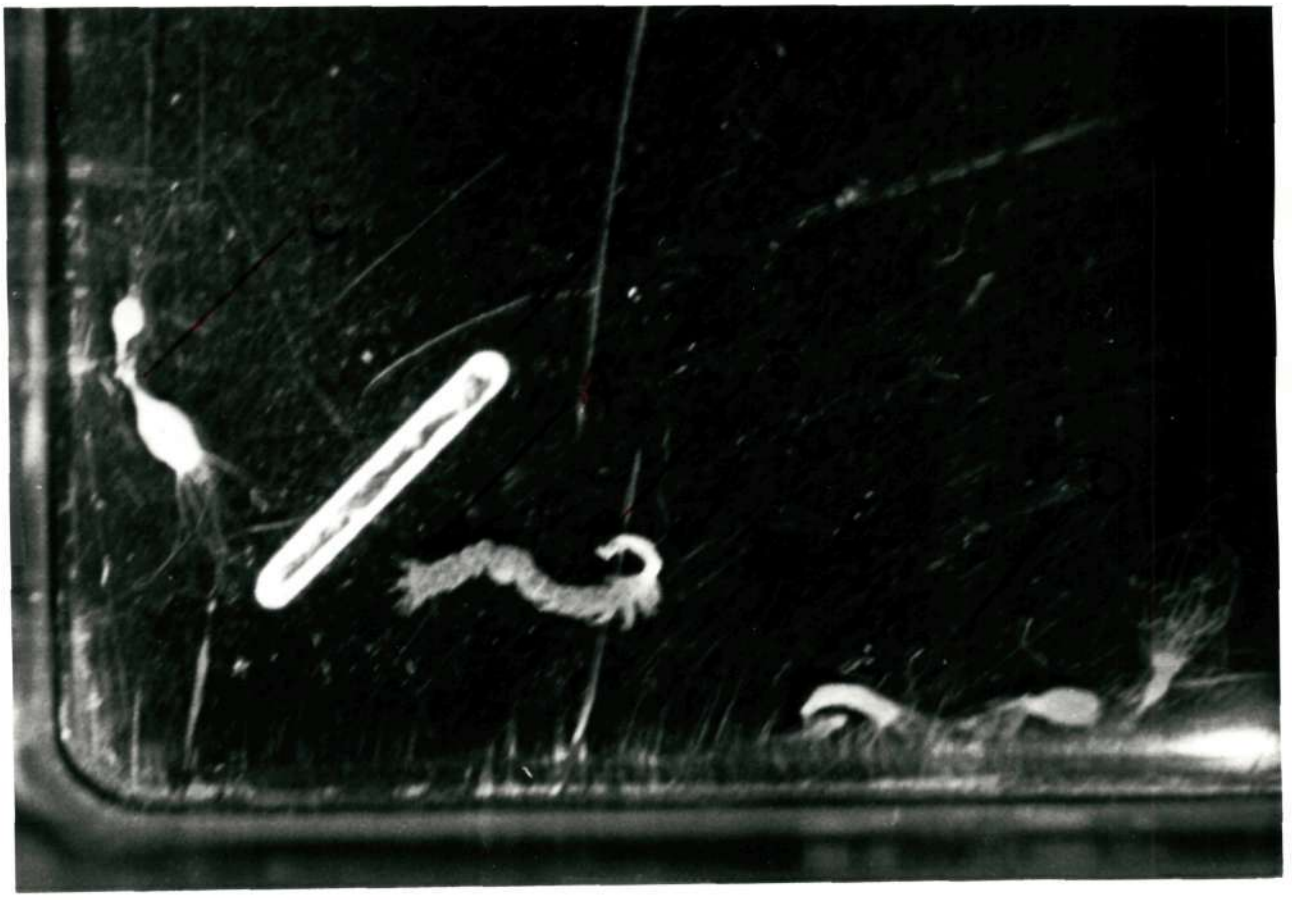

Figure 4

Polyps of Chrysaora quinquecirrha

These polyps are contained in 20‰ artificial sea water and were maintained at 20°C for 13 weeks before raising the temperature to 26°C. Following this temperature rise, one polyp only began to strobilate (A), demonstrating the asynchrony of the phenomenon. The podocyst end of this polyp is also indicated (B). In addition to the strobilating polyp, this test environment also contains two polyps attached to a common podocyst (C) as well as several single polyps (D). Structure (E) is part of the container.

observed to increase significantly in the presence of added phosphate or nitrate. In contrast, ammonium, sewage effluent or combinations of phosphate plus nitrate plus ammonium are detrimental to polyps. At low concentrations, these pollutants prevent polyp reproduction whereas at higher concentrations polyps die and disintegrate. It is of interest that phosphate and nitrate appear to exert a protective effect against the detrimental ammonium effect.

To test the validity of our results suggesting that polyps proliferate more rapidly in the presence of added phosphate or nitrate, a second experiment was initiated in which selected concentrations of either phosphate (0.01, 0.05, 0.25 ppm phosphorus) or nitrate (10, 30 ppm nitrogen) was added to 20‰ artificial sea water and compared to four controls lacking added pollutants. Each 25 ml test environment contained eight polyps and was examined in duplicate and at three temperatures (5°, 20°, and 26°C). In addition, for one concentration of either phosphate (0.05 ppm-P) or nitrate (10 ppm-N), polyps initially with and without podocysts were compared.

The total duration of this experiment was 17 weeks (the time remaining until the end of the program). After

12 weeks, those polyps incubated at 5° were raised to 20° and after an additional two weeks, these polyps were raised to 26°. Polyps initially maintained at 20°C were raised to 26° after the 14th week whereas no temperature changes were made to those polyps initially maintained at 26°C. Experimental maintenance was essentially as described for the first experiment except that for each environmental situation weekly records were maintained for both polyp numbers (Tables 10 through 12) and podocyst numbers (Tables 13 through 15).

The results (not shown) for maintenance at 5° are in agreement with those previously obtained. Thus, all polyps shrank in size within one week of exposure to this temperature and no differences were observed between control and test environments. Although the temperature was raised after 12 weeks of maintenance, no polyps had yet formed by the termination of the experiment. However, probably insufficient time had elapsed for their appearance.

At 26°C, pollution-free control polyps show an average increase in number from eight to ten although considerable variation is observed among each control medium (Table 10). The initial presence of a podocyst

TEMP	CONTROL #	± PODOCYSTS ON POLYPS	VIABLE POLYP COUNT AT END OF INDICATED WEEK																	
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
26°	1	-	8	8	-	8	8	9	9	8	8	-	-	7	8	8	7	-	7	7
	2	-	8	8	-	8	8	9	9	11	10	-	-	10	11	11	12	-	12	11
	3	-	8	8	-	8	10	10	12	11	12	-	-	15	15	15	13	-	12	13
	4	-	8	8	-	9	10	9	9	10	10	-	-	11	10	9	10	-	10	9
	5	+	8	8	9	9	-	-	10	10	10	10	-	10	10	-	-	-	-	-
	6	+	8	8	8	8	-	-	8	8	8	8	-	8	8	-	-	-	-	-
20°	1	-	8	8	-	8	8	8	8	8	8	-	-	8	8	8	8	-	8	8
	2	-	8	8	-	8	8	8	8	8	8	-	-	8	8	8	8	-	8	8
	3	-	8	8	-	8	8	8	8	8	8	-	-	8	8	8	8	-	8	8
	4	-	8	8	-	8	8	8	8	8	8	-	-	8	8	8	8	-	8	8
	5	+	8	8	8	8	-	-	9	8	9	9	-	9	9	-	-	-	-	-
	6	+	8	8	9	9	-	-	10	10	9	10	-	11	10	-	-	-	-	-

raise temperature to 21°C

Table 10

Survival and Multiplicity of Polyps With and Without Podocysts

At each of the indicated temperatures, 8 polyps were placed into each of 6 control environments containing approximately 25 ml of 20‰ artificial sea water at pH 7.8. In 4 of these environments the polyps initially lacked podocysts whereas in the other 2 environments polyps initially possessed podocysts. Fresh media were supplied weekly and polyps were fed twice weekly with brine shrimp. Polyps were observed for morphological changes and counted once a week. A temperature rise after 14 weeks is also indicated.

TEMP	ppm-P	± PODOCYSTS ON POLYPS	VIABLE POLYP COUNT AT END OF INDICATED WEEK																	
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
26°	0.01	-	8	8	-	8	8	8	8	7	7	-	-	7	7	7	7	-	7	9
	0.01	-	8	8	-	8	8	8	9	9	10	-	-	10	9	10	10	-	10	10
	0.05	-	8	8	-	8	8	8	8	8	8	-	-	8	8	8	8	-	8	8
	0.05	-	8	8	-	8	8	8	8	9	8	-	-	9	9	9	9	-	9	9
	0.25	-	8	8	-	8	8	9	9	11	12	-	-	13	12	13	11	-	12	12
	0.25	-	8	8	-	8	9	9	11	11	11	-	-	13	12	11	11	-	11	11
	0.05	+	8	8	-	8	10	12	11	12	14	-	-	13	13	14	15	-	12	15
	0.05	+	8	8	-	8	9	7	7	8	8	-	-	8	7	7	8	-	7	7
	0.05	+	8	8	8	8	-	-	10	10	11	10	-	10	10	-	-	-	-	-
	0.05	+	8	8	9	9	-	-	12	11	12	13	-	12	12	-	-	-	-	-
20°	0.01	-	8	8	-	8	8	8	8	8	8	-	-	9	8	9	7	-	8	7
	0.01	-	8	8	-	8	8	10	9	11	11	-	-	11	11	11	11	-	11	11
	0.05	-	8	8	-	8	8	8	9	6	6	-	-	6	6	6	6	-	6	6
	0.05	-	8	8	-	8	8	8	8	8	8	-	-	8	8	8	7	-	8	8
	0.25	-	8	8	-	8	9	9	8	8	8	-	-	8	9	9	9	-	9	9
	0.25	-	8	8	-	8	8	8	9	8	8	-	-	7	7	7	7	-	7	7
	0.05	+	8	8	-	10	11	11	11	11	11	-	-	11	11	11	11	-	9	10
	0.05	+	8	8	-	8	10	9	9	10	10	-	-	10	9	9	8	-	9	10
	0.05	+	8	8	8	8	-	-	9	8	8	8	-	8	9	-	-	-	-	-
	0.05	+	8	8	7	7	-	-	10	10	10	9	-	9	9	-	-	-	-	-

raise temperature to 26°C

Table 11

Effect of Phosphate on Survival and Multiplicity of Polyps With and Without Podocysts

At each of the indicated temperatures, 8 polyps were placed into each of 10 test environments containing approximately 25 ml of 20‰ artificial sea water to which had been added the indicated amount of phosphate (as ppm phosphate phosphorus). The pH of each medium was maintained at 7.8. The initial presence or absence of podocysts on the polyps is indicated. Maintenance conditions are as described for Table 10.

TEMP	ppm-N	± PODOCYSTS ON POLYPS	VIABLE POLYP COUNT AT END OF INDICATED WEEK																	
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
26°	10	-	8	8	-	9	12	9	11	11	10	-	-	11	11	10	11	-	11	11
	10	-	8	8	-	8	8	8	8	8	8	-	-	8	8	8	8	-	8	7
	30	-	8	8	-	8	10	9	11	10	11	-	-	11	10	11	10	-	11	11
	30	-	8	8	-	8	8	8	10	9	9	-	-	11	11	11	11	-	12	11
	10	+	8	8	-	8	8	8	12	10	10	-	-	11	10	11	10	-	10	11
	10	+	8	8	-	10	10	8	10	10	10	-	-	10	10	10	10	-	9	8
20°	10	-	8	8	-	8	8	8	8	8	8	-	-	9	8	8	8	-	8	8
	10	-	8	8	-	8	8	8	8	8	8	-	-	8	8	8	8	-	8	8
	30	-	8	8	-	8	7	7	6	6	6	-	-	6	6	6	6	-	6	6
	30	-	8	8	-	8	8	7	7	7	7	-	-	7	7	7	7	-	7	7
	10	+	8	8	-	8	8	8	6	7	6	-	-	6	6	6	6	-	6	6
	10	+	8	8	-	9	8	10	10	9	10	-	-	10	11	10	11	-	10	10

raise temperature to 26°C

Table 12

Effect of Nitrate on Survival and Multiplicity of Polyps With and Without Podocysts

At each of the indicated temperatures, 8 polyps were placed into each of 6 test environments containing approximately 25 ml of 20‰ artificial sea water to which had been added the indicated amount of nitrate (as ppm nitrate nitrogen). The pH of each medium was maintained at 7.8. The initial presence or absence of podocysts on the polyps is indicated. Maintenance conditions are as described for Table 10.

TEMP	CONTROL #	± PODOCYSTS ON POLYPS	PODOCYST COUNT AT END OF INDICATED WEEK																
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
26°	1	-	0	-	-	-	-	7	9	5	7	-	-	6	8	8	7	-	6	8
	2	-	0	-	-	-	-	6	9	10	10	-	-	10	11	11	12	-	11	11
	3	-	0	-	-	-	-	9	12	11	12	-	-	15	15	15	12	-	12	15
	4	-	0	-	-	-	-	7	9	7	7	-	-	10	10	8	10	-	10	9
	5	+	8	-	-	-	-	-	10	10	10	10	-	10	10	-	-	-	-	-
	6	+	8	-	-	-	-	-	8	8	8	8	-	8	8	-	-	-	-	-
20°	1	-	0	-	-	-	-	8	8	8	8	-	-	8	8	8	8	-	8	8
	2	-	0	-	-	-	-	5	4	4	4	-	-	5	6	7	6	-	6	7
	3	-	0	-	-	-	-	3	3	4	4	-	-	3	5	5	5	-	5	6
	4	-	0	-	-	-	-	7	6	7	6	-	-	7	5	7	5	-	7	8
	5	+	8	-	-	-	-	-	9	8	9	9	-	9	9	-	-	-	-	-
	6	+	8	-	-	-	-	-	10	10	9	10	-	11	10	-	-	-	-	-

raise temperature to 26°C

Table 13

Comparison of Podocyst Formation on Polyps Initially With and Without Podocysts

Eight polyps were initially present in each control environment and the initial presence or absence of podocyst material is indicated. Conditions and polyp numbers at each week are given in Table 10. After 5 weeks of maintenance, podocyst counts were taken weekly.

TEMP	ppm-P	± Podocysts on Polyps	Podocyst count at end of indicated week																	
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
26°	0.01	-	0	-	-	-	-	8	8	7	7	-	-	7	7	6	6	-	7	8
	0.01	-	0	-	-	-	-	5	9	7	8	-	-	9	8	8	9	-	9	9
	0.05	-	0	-	-	-	-	5	5	5	5	-	-	6	5	6	7	-	7	7
	0.05	-	0	-	-	-	-	4	4	6	5	-	-	7	5	7	5	-	6	7
	0.25	-	0	-	-	-	-	6	6	6	8	-	-	11	9	10	9	-	11	10
	0.25	-	0	-	-	-	-	6	7	7	6	-	-	11	10	10	8	-	10	10
	0.05	+	8	-	-	-	-	12	11	12	14	-	-	13	13	14	15	-	12	15
	0.05	+	8	-	-	-	-	7	7	8	8	-	-	8	7	7	8	-	7	7
	0.05	+	8	-	-	-	-	-	10	10	11	10	-	10	10	-	-	-	-	-
	0.05	+	8	-	-	-	-	-	12	11	12	13	-	12	12	-	-	-	-	-
20°	0.01	-	0	-	-	-	-	7	6	6	7	-	-	8	7	7	6	-	7	6
	0.01	-	0	-	-	-	-	9	7	8	10	-	-	10	10	9	10	-	10	11
	0.05	-	0	-	-	-	-	7	5	2	2	-	-	2	2	2	4	-	4	5
	0.05	-	0	-	-	-	-	7	7	7	7	-	-	7	7	7	7	-	7	7
	0.25	-	0	-	-	-	-	9	8	8	8	-	-	8	8	9	9	-	8	9
	0.25	-	0	-	-	-	-	7	6	5	6	-	-	6	7	6	7	-	7	7
	0.05	+	8	-	-	-	-	11	11	11	11	-	-	11	11	11	11	-	9	11
	0.05	+	8	-	-	-	-	9	9	10	10	-	-	10	9	9	8	-	9	9
	0.05	+	8	-	-	-	-	-	9	8	8	8	-	8	9	-	-	-	-	-
	0.05	+	8	-	-	-	-	-	10	10	10	9	-	9	9	-	-	-	-	-

Raise temperature to 24°C.

Table 14

Effect of Phosphate on Podocyst Formation

Eight polyps were initially present in each test environment containing the indicated amount of phosphate (as ppm-phosphorus). The initial presence or absence of podocyst material is indicated. Conditions and polyp numbers at each week are given in Table 11. After 5 weeks of maintenance, podocyst counts were taken weekly.

TEMP	ppm-N	± PODOCYSTS ON POLYPS	PODOCYST COUNT AT END OF INDICATED WEEK																	
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
26°	10	-	0	-	-	-	-	6	7	8	8	-	-	8	8	5	8	-	9	7
	10	-	0	-	-	-	-	7	6	8	6	-	-	8	8	8	7	-	8	7
	30	-	0	-	-	-	-	7	8	9	8	-	-	10	9	10	10	-	11	11
	30	-	0	-	-	-	-	7	8	8	7	-	-	11	10	11	11	-	12	10
	10	+	8	-	-	-	-	8	12	10	10	-	-	11	10	11	10	-	10	11
	10	+	8	-	-	-	-	8	10	10	10	-	-	10	10	10	10	-	9	9
20°	10	-	0	-	-	-	-	6	5	5	4	-	-	5	4	5	4	-	4	4
	10	-	0	-	-	-	-	4	4	4	4	-	-	4	5	5	5	-	6	5
	30	-	0	-	-	-	-	5	3	5	4	-	-	5	4	4	5	-	4	5
	30	-	0	-	-	-	-	5	5	5	4	-	-	6	6	6	6	-	5	3
	10	+	8	-	-	-	-	8	6	7	6	-	-	6	6	6	6	-	6	6
	10	+	8	-	-	-	-	10	10	9	10	-	-	10	11	10	11	-	10	10

raise temperature to 26°C

Table 15

Effect of Nitrate on Podocyst Formation

Eight polyps were initially present in each test environment containing the indicated amount of nitrate (as ppm-nitrogen). The initial presence or absence of podocyst material is indicated. Conditions and polyp numbers at each week are given in Table 12. After 5 weeks of maintenance, podocyst counts were taken weekly.

on the polyp does not seem to effect significantly this rate of increase at 26°. In the presence of either added phosphate (Table 11) or nitrate (Table 12), polyps at 26° increase at about the same rate as the controls and no differences are observed between polyps initially possessing or lacking podocysts.

At 20°C, pollution-free controls lacking podocysts are healthy but do not increase in number (Table 10). However, polyps at this temperature which initially have podocysts do multiply and the rate of increase is about the same as that seen at 26°C. In the presence of added phosphate (Table 11), polyps initially with or without podocysts increase in number at about the same rate and this rate is not significantly different from that seen in controls possessing podocysts. In the presence of added nitrate (Table 12), those polyps lacking podocysts do not multiply whereas one group with podocysts multiplied at about the same rate as the controls with podocysts.

Thus, in this experiment, phosphate and nitrate are not detrimental to polyps, but the dramatic increase in polyp numbers which was observed in the first experiment in the presence of these pollutants did not occur. There is some evidence (Table 11), however, that phosphate may

contribute to the proliferation of polyps, especially polyps lacking podocysts and maintained at lower temperatures (20°). The results are also indicative that complex interrelationships exist between podocyst material, temperature, and added pollutants in regulating the multiplicity of polyps.

It became apparent after about five weeks of maintenance of this experiment that many polyps originally lacking podocysts developed podocysts. This development was essentially complete by five weeks although some small increases in podocyst number occurred after this time (Tables 13 through 15). For those polyps originally lacking podocysts, not all polyps later developed podocysts. For those polyps originally possessing podocysts, all polyps were attached to podocyst material and the podocyst count is presented as identical to the viable polyp count. However, podocyst counts do not necessarily match viable polyp counts because two or three polyps often grow from a common podocyst. Thus, although these data are subject to certain ambiguities, it may be said that, for polyps originally lacking podocysts, pollution-free controls probably developed more podocysts at 26° than at 20° (Table 13). The presence of added nitrate (Table 15) made little difference to the number of podocysts formed

whereas added phosphate (Table 14) appeared to enhance podocyst formation somewhat. Thus, these data tend to support the findings of the first experiment that phosphate appears to contribute to sea nettle proliferation.

Upon raising the temperature of those polyps maintained at 20° for 14 weeks, some polyps began to strobilate. Strobilation was again asynchronous, however, and no significant differences in frequency were observed among the various groups. No polyps maintained only at 26°C strobilated. These data are in agreement with those of the first experiment.

2. Effect of Polyps on Pollutant Concentrations

To complement our maintenance studies reported in the preceeding section, we conducted a study to determine the effect of polyps on the concentration of the pollutant in the test environments. Toward the end of our first maintenance experiment (described in the preceeding section), an aliquot of each fresh medium was saved for comparison with the same medium after one week of polyp contact. Colorimetric methods were utilized to test for phosphate by stanous chloride and ammonium molybdate (11), nitrate by the Brucine method (12), nitrite by sulfanilic acid and N-(napthyl) ethylenediamine dihydrochloride (13), and ammonium by the Phenate method (14).

The results show an increase in the phosphate concentration during one week in contact with the polyps (Table 16). The amount of increase is fairly constant in each phosphate concentration so that the percentage increase diminishes as the initial phosphate concentration increases. The experimental design requires that the test environment of the polyps be changed once weekly and that during the week, polyps are fed brine shrimp daily. Appropriate controls have established that this additional phosphate comes from the daily addition of brine shrimp to the test environment rather than from the polyps (Table 17). Thus, it may be concluded that the initial phosphate concentration of each test environment is the minimum concentration to which polyps are exposed each week and that polyps per se do not significantly change this concentration.

Table 18 shows that the nitrate concentration also increases during one week of contact with the polyps. This increase is similar to that seen with phosphate since it is more significant at the lower initial concentrations than at the higher concentrations. Appropriate controls have established that this additional nitrate likewise originates from the daily brine shrimp feeding and that

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Table 16

Phosphate Concentration of Test Environments
Before and After One Week of Contact with Polyps

Theoretical Initial Phosphorus Content of Test Environment (mg/l)	Time Tested	Average mg/l
None	Before Feeding	0.00
None	After Feeding	0.08
0.05	Before Feeding	0.06
0.05	After Feeding	0.23
0.25	Before Feeding	0.26
0.25	After Feeding	0.33
1.00	Before Feeding	1.03
1.00	After Feeding	1.77
5.00	Before Feeding	5.20
5.00	After Feeding	5.70
20.00	Before Feeding	22.60
20.00	After Feeding	23.00

Polyps were placed in a fresh test environment containing the indicated concentration of phosphate. Aliquots were saved from each medium. During the following week, polyps were fed daily with brine shrimp contained in a small amount of 20‰ artificial sea water. At the end of this week, the phosphate concentration of aliquots of the test environment taken before and after polyp contact was tested by the stanous chloride and ammonium molybdate method (11).

Table 17

Comparative Effect of Polyps, Brine Shrimp, and Brine Shrimp Water on Phosphate Concentration of Medium

Theoretical Initial Phosphate Concentration (ppm-phosphorus)	Additions to Medium for One Week of Contact	Tested Average ppm-P After One Week Contact
0	None	0
	Polyps only	0.02
	Brine Shrimp + Brine Shrimp Water	0.06
	Brine Shrimp Water	0
1.0	None	1.02
	Polyps only	1.03
	Brine Shrimp + Brine Shrimp Water	1.20
	Brine Shrimp Water	1.10

Fresh polyp medium was prepared consisting of 20‰ artificial sea water to which was added the indicated amount of phosphate phosphorus. Each medium was divided into four portions and additions made to each portion as indicated. At the end of one-week, each portion of each medium was tested for phosphate phosphorus content according to the stanous chloride and ammonium molybdate method (11).

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Table 18

Ammonium Concentration of Test Environments
Before and After One Week of Contact with Polyps

Theoretical Initial Nitrogen Content of Test Environment (mg/l)	Time Tested	Average mg/l
None	Before Feeding	< 0.1
None	After Feeding	< 0.1
1.0	Before Feeding	0.9
1.0	After Feeding	< 0.1
3.0	Before Feeding	3.0
3.0	After Feeding	< 0.1
10.0	Before Feeding	10.4
10.0	After Feeding	0.3
30.0	Before Feeding	31.8
30.0	After Feeding	27.0
100.0	Before Feeding	108.0
100.0	After Feeding	114.0

Polyps were placed in a fresh test environment containing the indicated concentration of ammonium nitrogen. Aliquots were saved from each medium. During the following week polyps were fed daily with brine shrimp contained in a small amount of 20‰ artificial sea water. At the end of this week, the nitrogen concentration of aliquots of the test environment taken before and after polyp contact was tested by the Phenate method (14).

polyps per se do not affect the nitrate concentration. It is of interest that the nitrite concentrations of these media also similarly increase during the week as a result of feeding.

Similar studies have been conducted to test the effect of polyps on the ammonium concentration of the test environment (Table 19). In contrast to the results obtained with phosphate and nitrate, the results with ammonium show a dramatic decrease in the ammonium concentration during one week of contact with polyps. This decrease occurs only at the lower initial ammonium concentrations (0 - 10 ppm nitrogen) and not for initial concentrations of 30 or 100 ppm nitrogen. Appropriate controls established that ammonium disappearance results from polyp contact and not from the daily addition of brine shrimp to the test environment. This failure to observe a significant decrease in the ammonium concentration for initial concentrations of 30 or 100 ppm nitrogen must reflect the fact that the polyps in these test environments are dead and can no longer utilize the available ammonium. This is consistent with and supportive of the findings herein that ammonium is detrimental to polyps. It is of interest that nitrite and nitrate concentrations also increase with ammonium disappearance. It was not established, however, whether this increase was due to brine shrimp feeding or polyps per se.

Table 19

Nitrate Concentration of Test Environments
Before and After One Week of Contact with Polyps

Theoretical Initial Nitrogen Content of Test Environment (mg/l)	Time Tested	Average mg/l
None	Before Feeding	0.1
None	After Feeding	0.7
1.0	Before Feeding	1.0
1.0	After Feeding	2.8
3.0	Before Feeding	3.4
3.0	After Feeding	5.3
10.0	Before Feeding	10.0
10.0	After Feeding	10.4
30.0	Before Feeding	33.4
30.0	After Feeding	31.8
100.0	Before Feeding	100.0
100.0	After Feeding	106.0

Polyps were placed in a fresh test environment containing the indicated concentration of nitrate nitrogen. Aliquots were saved from each medium. During the following week, polyps were fed daily with brine shrimp contained in a small amount of 20‰ artificial sea water. At the end of this week, the nitrogen concentration of aliquots of the test environment taken before and after polyp contact was tested by the Brucine method (12).

3. Summary and Conclusions Regarding Maintenance Studies With and Without Pollutants

In summary of the experiments reported in these sections, the following conclusions may be made regarding maintenance of Chrysaora quinquecirrha polyps:

a) Effects at Constant Temperature

1) Pollution-free controls are easily maintained in 20‰ artificial sea water at 20° and 26° over long periods of time. Polyp activities are strongly temperature regulated. Polyp proliferation occurs at both 20° and 26° but is far more pronounced at 26°. Those polyps originally lacking podocysts develop podocysts, perhaps somewhat faster at 26° than at 20°. At low temperature (5°C) polyps shrink and encyst.

2) At 5°, no effects of effects of added pollutants in the environment are obvious on polyp maintenance.

3) Added phosphate and nitrate may contribute to the proliferation of polyps. While the data are not entirely consistent, dramatic increases in polyp numbers have been observed under certain conditions in the presence of each of these two pollutants, especially phosphate. This effect is most pronounced at 26° but has

also been observed at 20°C. There is also some evidence that phosphate may enhance podocyst formation and, hence, cause proliferation. Neither phosphate nor nitrate is significantly changed in concentration as a result of contact with polyps per se.

4) At 20° and 26°, added ammonium and synthetic sewage effluent are detrimental to polyp health and viability. At low concentrations of these pollutants, polyps survive but are prevented from multiplying. As the concentration increases, polyps shrivel, die, and disintegrate into pieces. The higher the pollutant concentration, the quicker and more effective this process. The lethality of ammonium appears confirmed by the observed disappearance of ammonium from the medium as a result of polyp contact until lethality occurs.

5) A combination of pollutants containing ammonium, phosphate, and nitrate is also detrimental to polyps. Comparing equal concentrations of ammonium in the presence and absence of added phosphate and nitrate, it was observed that phosphate and nitrate "protect" polyps against the detrimental effects of ammonium.

6) No effect of pH in the range of 6.2 - 7.8 was seen.

b) Effects of Raising the Temperature

1) Upon raising the temperature to 26° after long-term storage at 20°, pollution-free control polyps begin to multiply somewhat more rapidly. Further, about 10 - 15% of all those control polyps experiencing the temperature rise begin to strobilate. Although strobilation is asynchronous both with respect to numbers participating per environment and time of onset, the phenomenon is never observed among those polyps which never experienced a temperature rise.

2) Upon raising the temperature after long-term storage at 5°, pollution-free control polyps develop new polyps at about a 10 - 20% recovery rate. A small percentage of these polyps strobilate.

3) For polyps maintained first at 5°, phosphate and nitrate have no detectible effect on the rate or frequency of new polyp formation following the temperature rise. However, no polyp formation occurred at the high concentrations of the detrimental pollutants. Thus, ammonium and synthetic sewage effluent prevent winter survival.

4) For polyps maintained first at 20° and then raised to 26°, the effects of phosphate and nitrate in enchancing polyp multiplication become more noticeable. However, the detrimental effects of ammonium and synthetic sewage effluent were not noticeably enhanced by this temperature increase.

5) No effects of pollutants on strobilation of healthy polyps could be ascertained, possibly due to the low frequency and the observed asynchrony of the phenomenon.

B. Polyp Metabolism

To complement the maintenance studies described in the preceeding section, we have sought to develop a method whereby polyp metabolism could be directly measured. Such a measurement would supplement the morphological measurements made in the maintenance studies and could provide an additional standard to determine the effects of pollutants on polyps. Further, a relatively quick metabolic assay could prove useful in predicting the effects of pollutants on polyps.

Although metabolic investigations in the similar Hydra have followed oxygen consumption (15), this method seemed too cumbersome for our purposes. In this section, the development of a different type of metabolic assay for

polyp metabolism is described and results obtained with pollutants are presented.

1. Development of the Assay

The assay examined is similar to the Labeled Release Experiment developed by Biospherics for the coming Viking '75 Mission to Mars (16-18). Individual polyps were placed into 0.5 ml of a mixture containing 20‰ artificial sea water (Table 1) and ^{14}C-labeled organic substrates. The atmosphere above the reaction mixtures was then "gettered" to follow $^{14}CO_2$ evolution as a function of time. It was found that each polyp produced a detectible response although considerable variation was observed among different polyps. Better consistency between duplicate reactions was obtained by placing two polyps in each reaction mixture and by rigorously cleaning all the glass planchets in which the reaction mixtures were contained. Variations among different polyps were then considerably reduced although not completely eliminated.

In the initial development of this assay, the ^{14}C-labeled substrates tested were glucose, glycine, lactate, and formate, each present both singly and in combination at a concentration of 2.5×10^{-4}M. The substrate best utilized

was glucose, the magnitude of the response increasing with the glucose concentration. Consequently, for further development of this technique, uniformly labeled ^{14}C-glucose (1.0 μCi/40 μg/ml) was utilized as substrate. Concentrations of glucose were kept relatively low, however, since polyps maintained in glucose plus 20‰ artificial sea water began to look unhealthy after a few days.

Sterile medium in the absence of added polyps did not produce a labeled release response. However, the possibility existed that innoculation of the reaction mixture with polyps also introduced bacteria into the system. That this was in fact the case was shown by using a polyp-containing reaction mixture to innoculate tryptone glucose yeast agar plates prepared in 20‰ artificial sea water. The large numbers of bacteria which subsequently grew on the agar plates suggested that the labeled release response could reflect polyp metabolism, bacterial metabolism, or a combination of both. In attempting to determine the magnitude of the bacterial response, several experiments were conducted in which polyps were first killed by high salt, drying, or chopping. In each case, labeled responses were still quite high. Consequently, a study with antibiotics was undertaken to eliminate the bacterial interference from our assay for polyp metabolism.

The antibiotics initially selected for study were Tetracycline and Sulfanilamide, both of which have a broad spectrum of toxicity, Penicillin G which is toxic to Gram Positive bacteria, and Polymyxin B which is toxic to Gram Negative bacteria. The results (Figures 5 through 8) show that each antibiotic is effective in lowering the labeled release response seen with unsterile medium alone and all four antibiotics show a concentration effect with the highest concentration of each essentially eliminating the labeled release response. On a molar basis, the order of effectiveness of the antibiotics was: Penicillin G $\gg$ Polymyxin B $>$ Tetracycline $>$ Sulfanilamide. When these antibiotics were added singly to sterile medium containing two polyps (and their attendant bacteria), each lowered the evolution of labeled gas and the percent inhibition increased with the antibiotic concentration. The residual response did not solely reflect polyp metabolism, however, because aliquots taken from each medium at 28 hours yielded some colonies when plated on agar.

Additional experiments to eliminate the remaining bacterial response have been performed only with penicillin and polymyxin since polyps in the presence of either of these

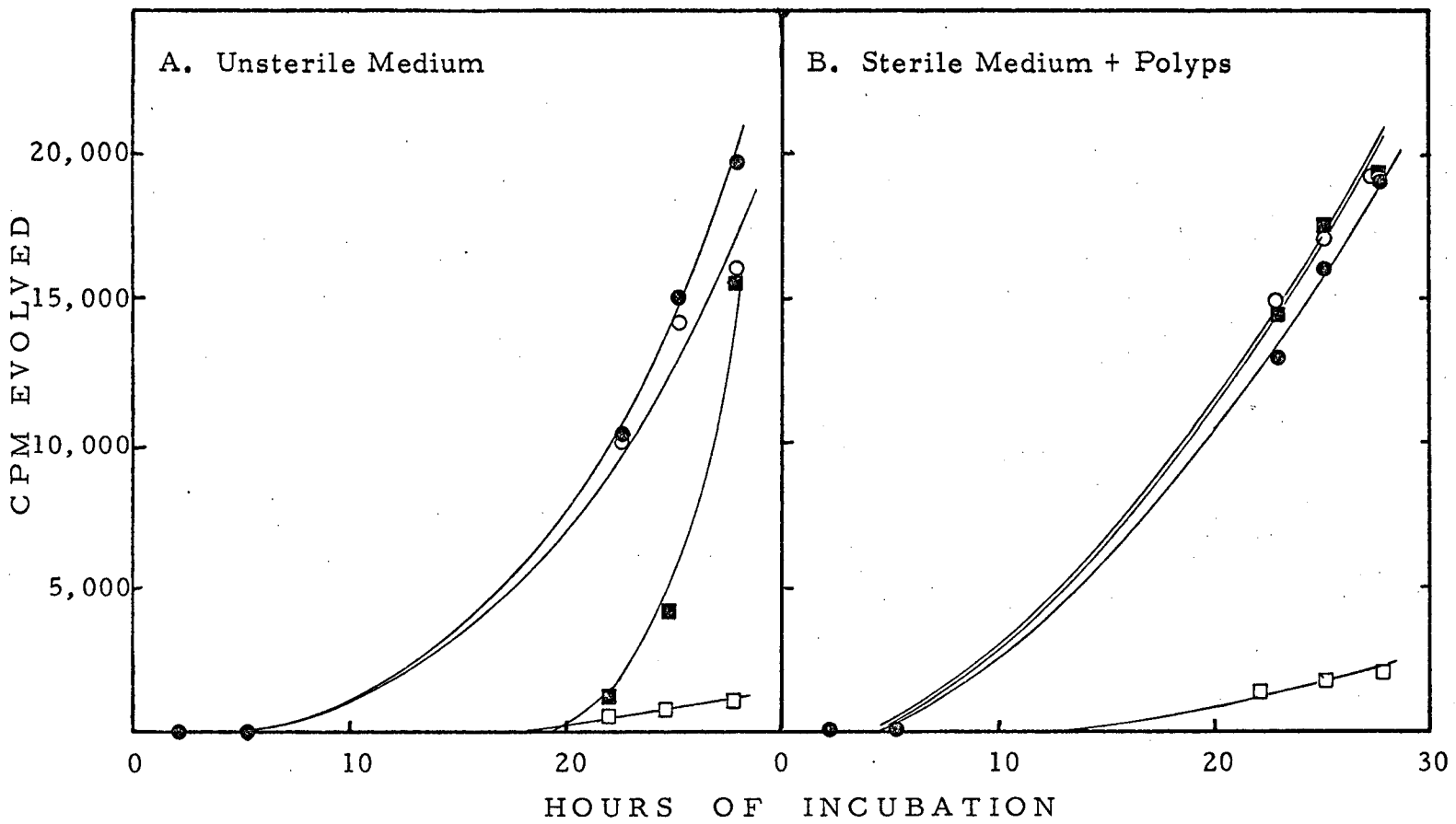


Figure 5

Effect of Tetracycline on Labeled Release from Unsterile Medium and from Sterile Medium with Polyps

Labeled Release in the presence and absence of varying amounts of the antibiotic Tetracycline HCl was compared over a 28 hour period for unsterile medium alone (A) and for sterile medium containing 2 polyps (B). Each 0.5 ml medium contained 20‰ artificial sea water, ^{14}C-glucose (1 μCi/40 μg/ml), and 0 (●——●), 0.37 μg (○——○), 3.75 μg (■——■), or 37.5 μg (□——□) of tetracycline. Assuming a molecular weight for tetracycline of 481, the highest antibiotic concentration is approximately 1.5×10^{-3}M.

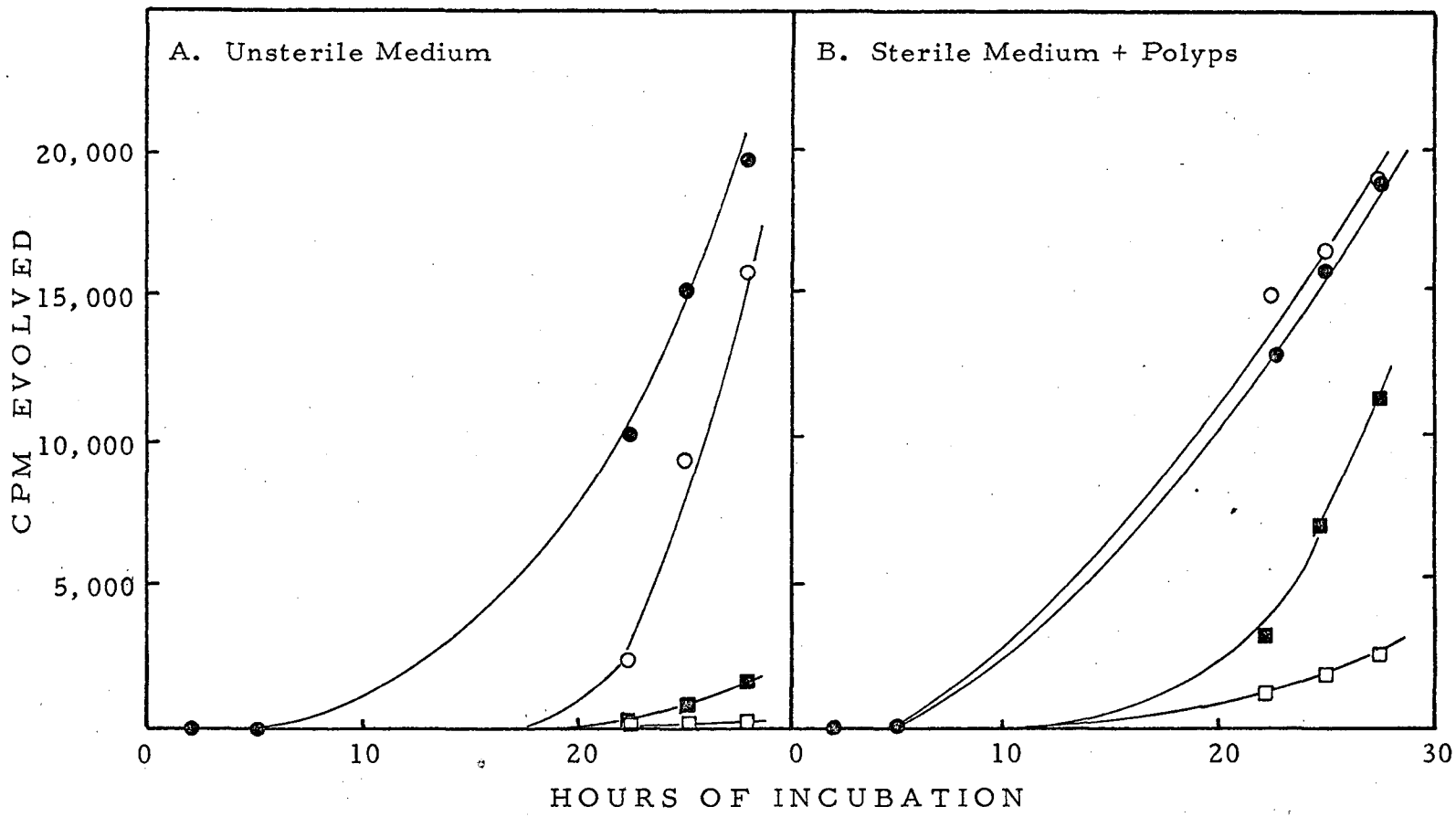


Figure 6

Effect of Sulfanilamide on Labeled Release from Unsterile Medium and from Sterile Medium with Polyps

Labeled Release in the presence and absence of varying amounts of the antibiotic Sulfanilamide was compared over a 28 hour period for unsterile medium alone (A) and for sterile medium containing 2 polyps (B). Each 0.5 ml medium contained 20‰ artificial sea water, ^{14}C-glucose (1 μCi/40 μG/ml), and 0 (●——●), 7 μg (○——○), 70 μg (■——■), or 700 μg (□——□) of sulfanilamide. Assuming a molecular weight for sulfanilamide of 172, the highest antibiotic concentration is approximately 10^{-2}M.

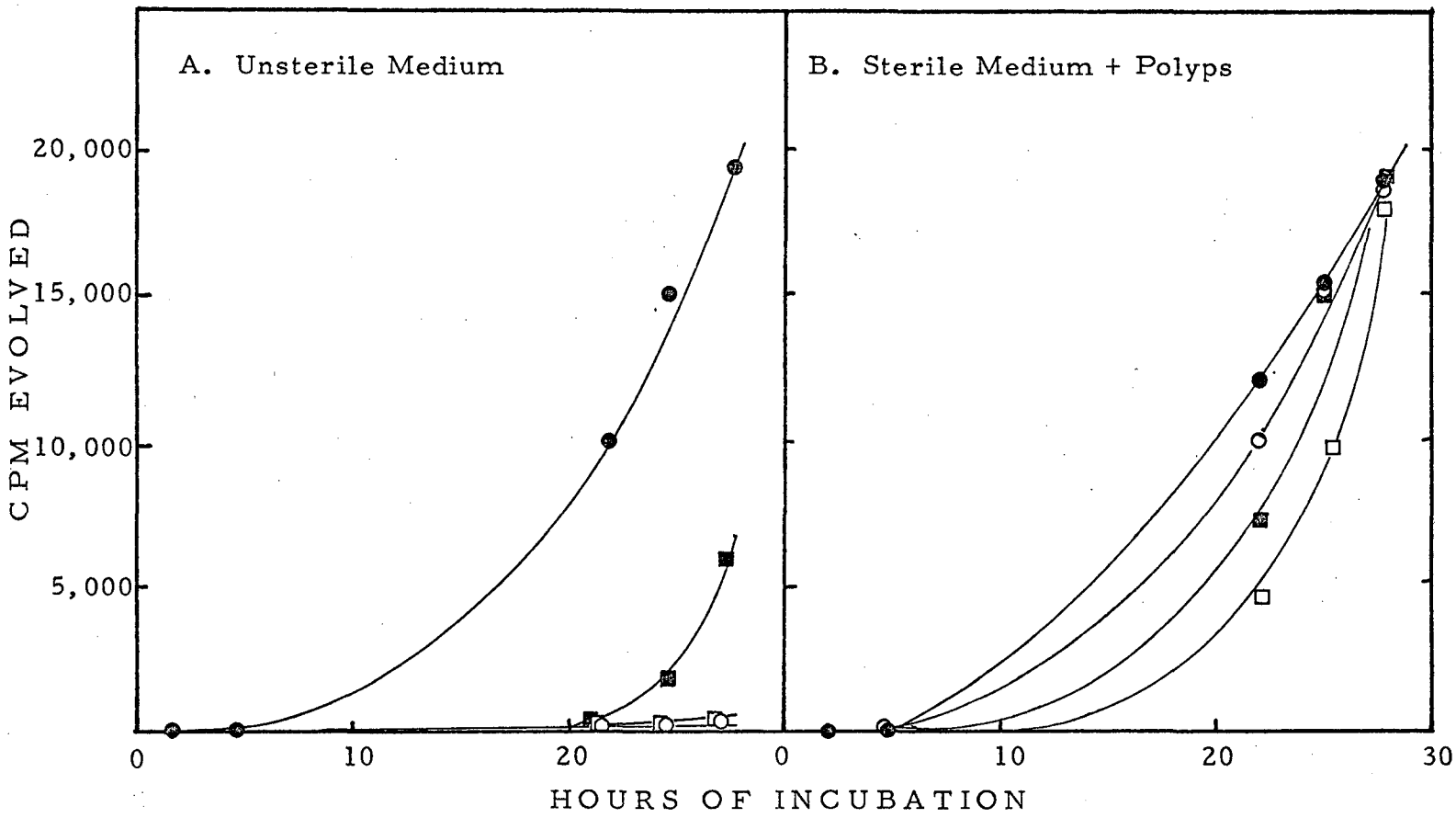


Figure 7

Effect of Penicillin G on Labeled Release from Unsterile Medium and from Sterile Medium with Polyps

Labeled Release in the presence and absence of varying amounts of the antibiotic Penicillin G was compared over a 28 hour period for unsterile medium alone (A) and for sterile medium containing 2 polyps (B). Each 0.5 ml medium contained 20‰ artificial sea water, ^{14}C-glucose (1 μCi/40 μg/ml) and 0 (●—●), 0.22 units (○—○), 2.2 units (■—■), or 22 units (□—□) of penicillin. Assuming that penicillin has 1435 units/mg and a molecular weight of 372, the highest antibiotic concentration is approximately 10^{-4}M.

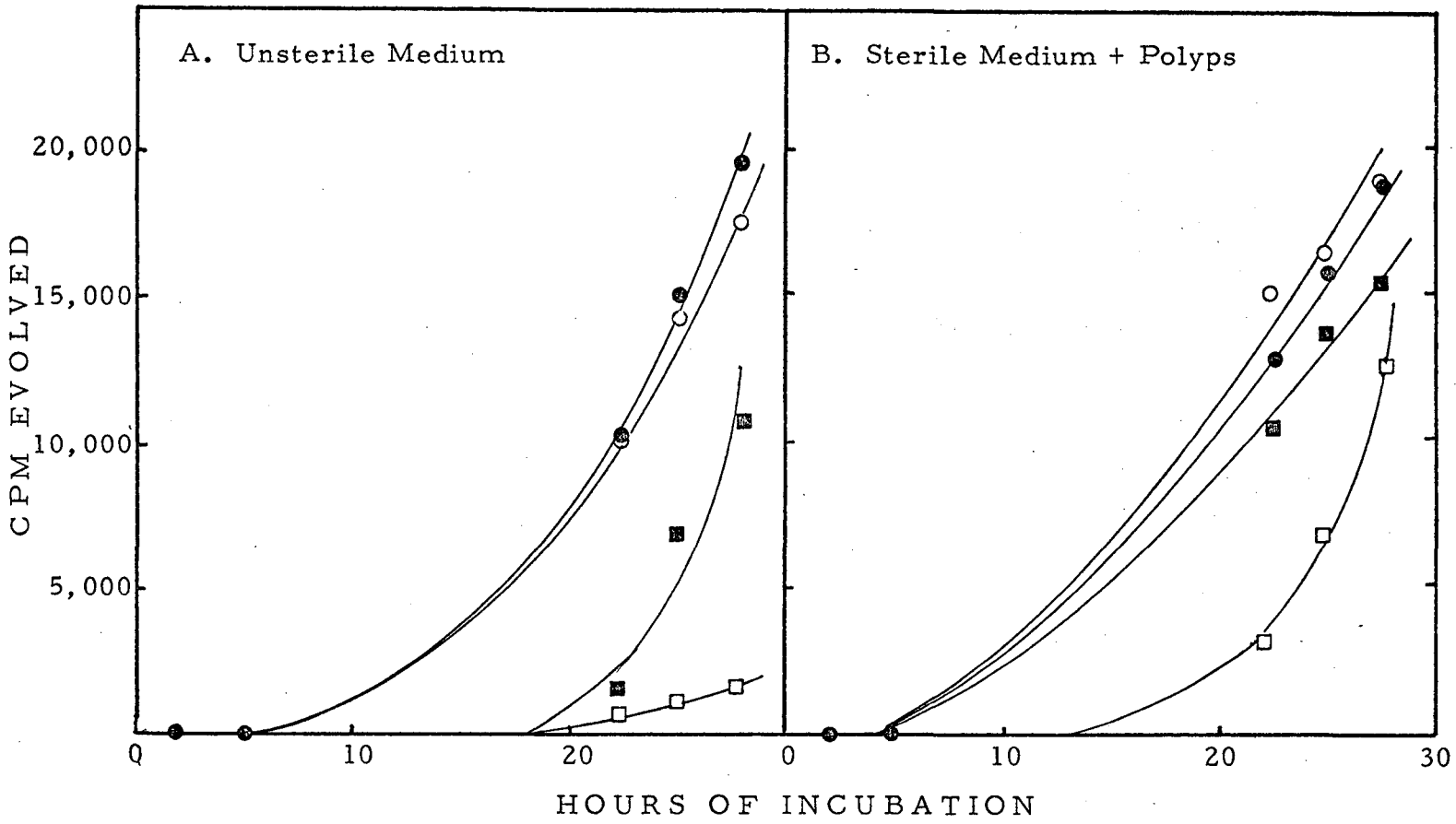


Figure 8

Effect of Polymyxin B on Labeled Release from Unsterile Medium and from Sterile Medium with Polyps

Labeled Release in the presence and absence of varying amounts of the antibiotic Polymyxin B Sulfate was compared over a 28 hour period for unsterile medium alone (A) and for sterile medium containing 2 polyps (B). Each 0.5 ml medium contained 20‰ artificial sea water, ^{14}C-glucose (1 μCi/40 μg/ml) and 0 (●—●), 0.15 μg (○—○), 1.5 μg (■—■), or 15 μg (□—□) of polymyxin. Assuming a molecular weight for polymyxin of 1380, the highest antibiotic concentration is approximately 2 x 10^{-5} M.

two antibiotics appeared healthy at the end of the 30-hour reaction discussed above whereas polyps in the presence of either sulfanilamide or tetracycline did not. Our results (Figure 9) indicate that a combination of both Penicillin G (440 units/ml medium) and Polymyxin B (300 μg/ml medium) will essentially eliminate the labeled release response from unsterile medium and also eliminate most of the responses from sterilized medium containing polyps. Further, when both media (with and without polyps) were plated on agar, absolutely no bacterial growth resulted. Thus, the small residual response seen in the presence of polyps and both antibiotics essentially represents polyp metabolism only. That these concentrations of penicillin and polymyxin are not detrimental to polyps was established by growing polyps in the presence of these antibiotics. After one week of maintenance, no effect was apparent.

The combination of penicillin and polymyxin was also compared to Streptomycin and to iodine as bactericidal agents. The results showed iodine to be completely unsatisfactory even when polyps were preliminary incubated with iodine before the addition of the labeled substrate to start the reaction. Streptomycin was better than iodine, but was not as effective

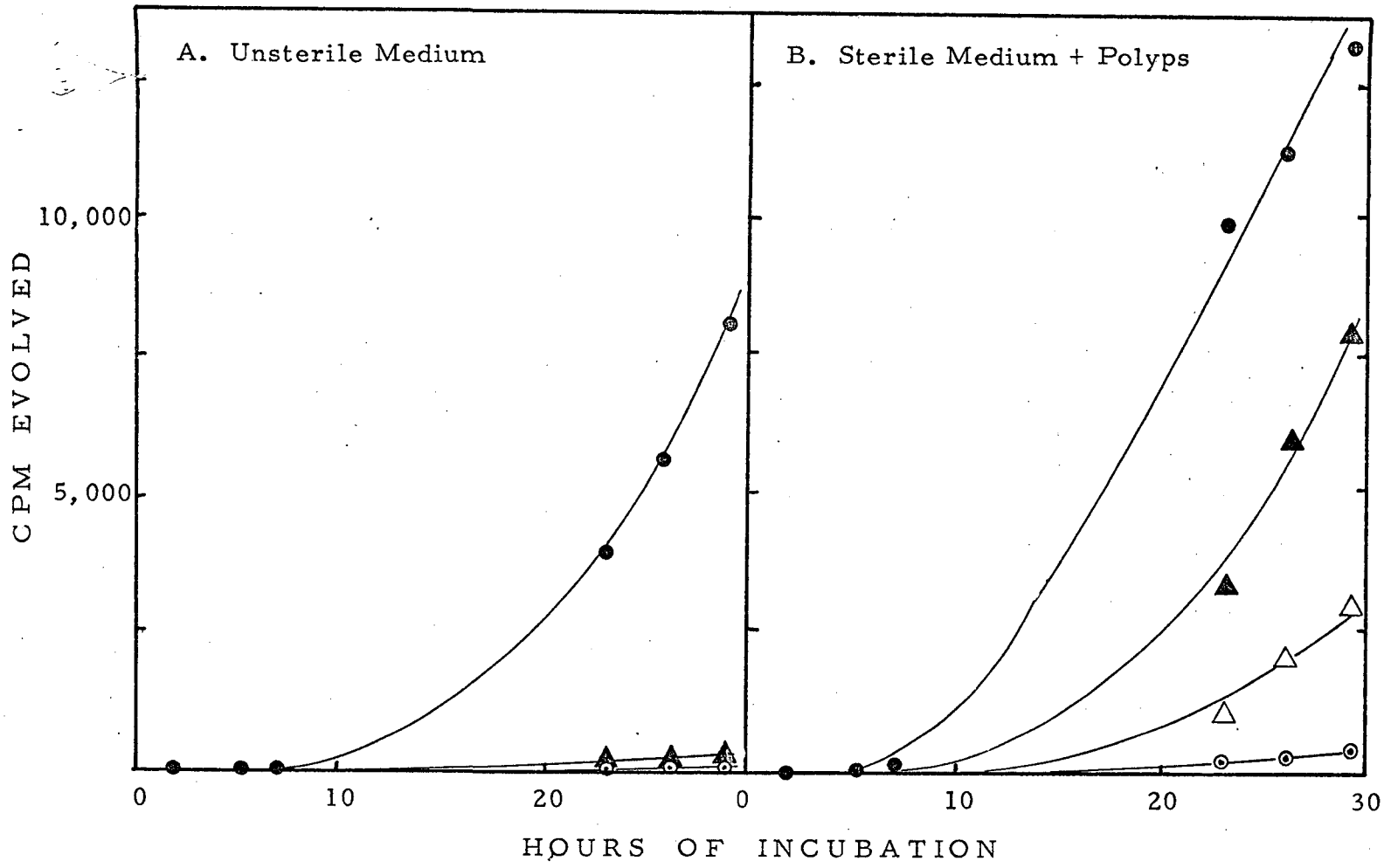


Figure 9

Effect of Combinations of Penicillin G and Polymyxin B on Labeled Release from Unsterile Medium and from Sterile Medium with Polyps

Labeled Release in the presence and absence of varying amounts of Penicillin G and Polymyxin B was compared over a 28 hour period for unsterile medium alone (A) and for sterile medium containing 2 polyps (B). Each 0.5 ml medium contained 20‰ artificial sea water and ^{14}C-glucose (1 μCi/40 μg/ml). Combinations of penicillin and polymyxin, respectively, were 2.2 units + 1.5 μg (▲—▲), 22 units + 15 μg (△—△), and 220 units + 150 μg (⊙—⊙). These combinations were compared to responses seen in the absence of added antibiotics (●—●).

as the combination of polymyxin and penicillin. Consequently, all additional experiments were conducted in the presence of these two antibiotics.

After about 24 hours of monitoring in the presence of penicillin and polymyxin, bacterial contamination often became evident when the reaction mixture was plated on agar. This contamination probably represented bacteria which were resistent to the antibiotic and which began to grow out at this time. Consequently, in order to ensure that our labeled release data reflected only polyp metabolism, we initiated a technique whereby aliquots of each labeled release mixture were removed at various times throughout the reaction and plated on agar to test for bacterial contamination. By utilizing this technique, we were able to establish when the data obtained by labeled release were an accurate representation of polyp metabolism and when they began to reflect bacterial metabolism as well.

Further optimization of this assay for monitoring polyp metabolism required an investigation of the effectiveness of various organic substrates. Using two polyps in each reaction mixture, concentration curves were obtained for glycine, alanine, glucose, or lactate as substrate (Figure 10).

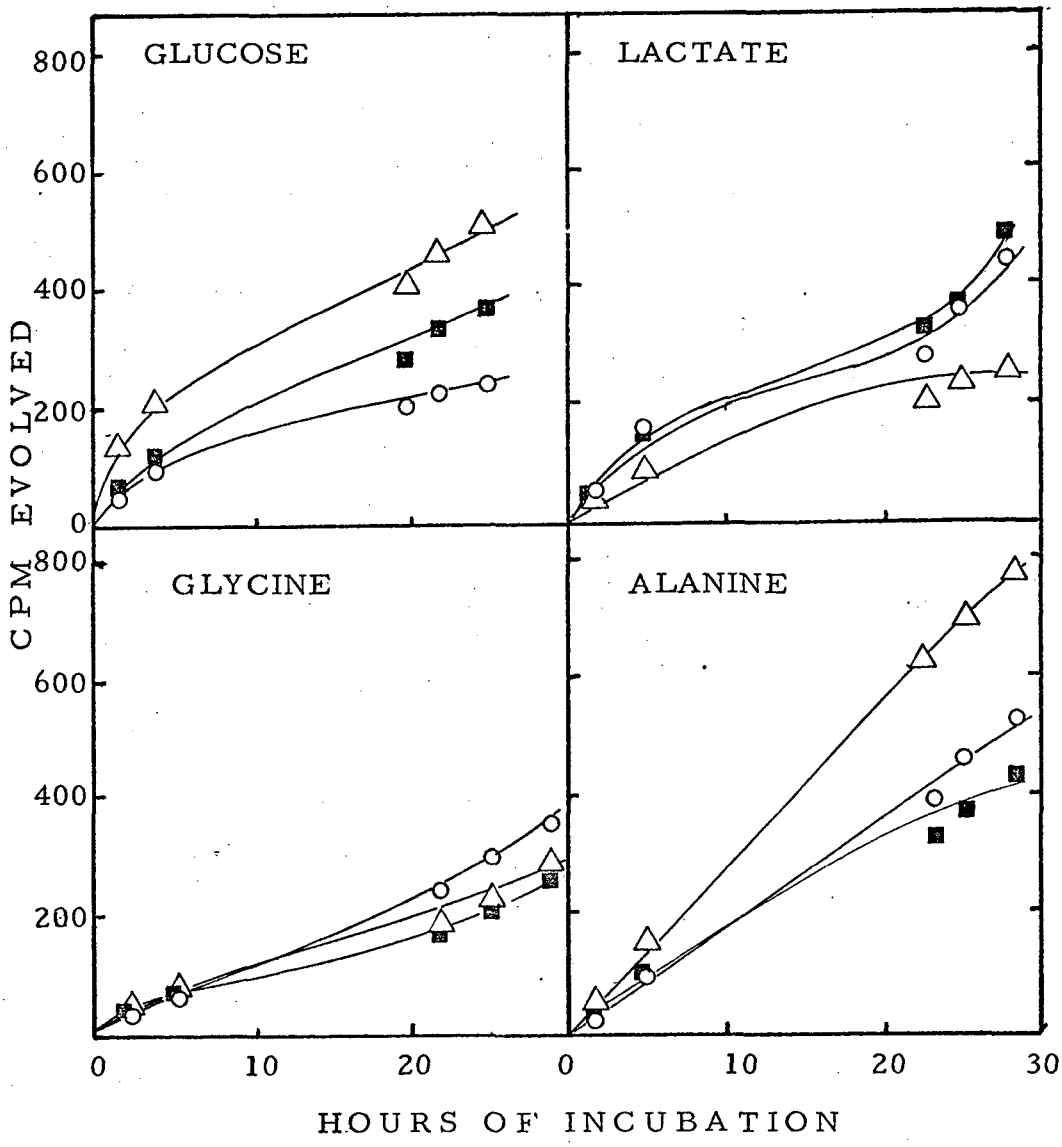


Figure 10

Comparison of Various Organics as Substrates for Polyp Metabolism

Each 1.0 ml reaction mixture contained 20‰ artificial sea water, 440 units of Penicillin G, 300 µg of Polymyxin B, and 2 polyps. In addition, ^{14}C-glucose, lactate, glycine, and alanine were each added separately, as indicated, at a final concentration of 2 µmoles/ml (o—o), 4 µmoles/ml (■—■), or 8 µmoles/ml (△—△). Each substrate was uniformly labeled at a specific activity of 1 µCi/ 4 umoles of carbon. The evolved counts from each medium were monitored for approximately 30 hours.

Each substrate was uniformly labeled and at a specific activity of 1 μCi/4 μmole carbon atom of the substrate. The results clearly show alanine and glucose to be the most effective substrates. Glycine and lactate, on the other hand, were not particularly effective. Both alanine and glucose showed increased gas evolution with increased substrate concentration whereas glycine did not show a concentration effect and lactate may even be inhibitory at higher concentrations. Additional experiments showed the combination of glucose plus alanine to be more effective than either substrate alone. Consequently, the substrates finally selected for this assay were the combination of glucose (12 μCi/8 μmoles/ml) and alanine (6 μCi/8 μmoles/ml).

In summary, then, the assay finally developed to monitor polyp metabolism contained two polyps in 0.5 ml of a medium containing penicillin (440 units/ml), polymyxin (300 μg/ml), ^{14}C-UL-glucose (12 μCi/8 μmoles/ml), ^{14}C-UL-L-alanine (6 μCi/8 μmoles/ml) in 20‰ artificial sea water. The reaction was "gettered" with a barium hydroxide saturated pad to collect the $^{14}CO_2$ evolved for at least 20 hours; periodically aliquots of 0.05 ml were also removed from each reaction

mixture and plated on agar to monitor for bacterial contamination.

2. Use of Assay to Monitor Effects of Pollutants

The labeled release assay has been utilized to examine the effect of various pollutants on polyps contained in medium both in the presence and absence of antibiotics. Experiments in the absence of antibiotics reflect the contribution of both polyps and contaminating bacteria and were conducted in an attempt to monitor the effect of pollutants on the metabolic state of the environment.

The pollutant chosen for careful investigation was ammonium chloride because of its established inhibitory effect on polyp maintenance (Table 7). In initial experiments lacking antibiotics, ammonium was present in the assay over the concentration range of 0 - 3000 ppm ammonium nitrogen. By using concentrations up to 30 times higher than those used in the maintenance studies, it was hoped to exaggerate any metabolic effect. However, the results (Figure 11) indicate no correlation between rate of gas evolution and ammonium concentration other than a random one. Preliminary incubation of the polyps with ammonium for 24 hours before the addition of labeled substrate to start the reaction did not significantly affect these results. Similarly, at concentrations

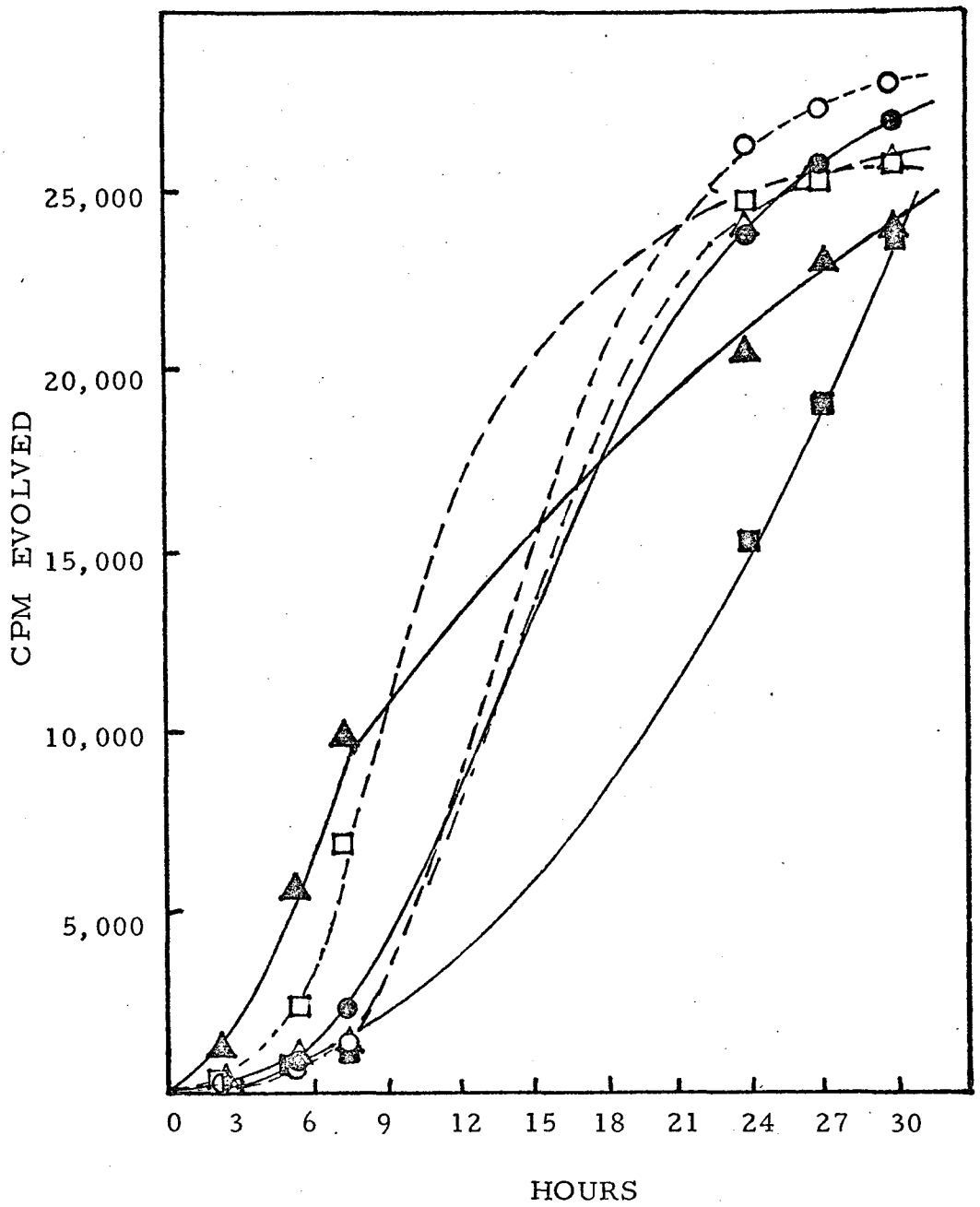


Figure 11

Effect of Ammonium Chloride on Labeled Release from Medium Containing Polyps. Two polyps were added to 0.5 ml of a medium containing 20 ‰ artificial sea water plus 0 (●—●), 30 (o---o), 100 (□---□), 300 (■——■), 1000 (△---△), or 3000 (▲——▲) ppm ammonium chloride nitrogen and pre-incubated for 24 hours. ^{14}C-labeled glucose was then added and the counts evolved measured for 30 hours. The counts evolved from each medium was negligible in the absence of polyps.

corresponding to those utilized in our maintenance studies, neither phosphate nor the combinations of phosphate plus nitrate plus ammonium produced a significant effect on labeled gas evolution in the absence of antibiotics.

With antibiotics present in the reaction mixture, the labeled gas evolved probably represents strictly polyp metabolism. Under these conditions, ammonium (Figure 12) does inhibit polyp metabolism at high concentrations (10 - 300 ppm-N) but not at the lowest concentrations examined (3 ppm-N). These results are in complete agreement with our findings of the effect of ammonium on polyp morphology (Table 7) and indicate the potential value of the technique as a monitor of pollutant effects.

Phosphate and nitrate have also been studied in the labeled release assay containing both polyps and antibiotics. The results with phosphate (Figure 13) show that polyp metabolism is somewhat lowered in the presence of all phosphate concentrations examined (0.5 - 20 ppm phosphorus). However, there is no direct relationship between the amount of gas evolved and the phosphate concentration. Thus, it may be concluded that added phosphate in this range is either not inhibitory to polyp metabolism, or that, if it is inhibitory,

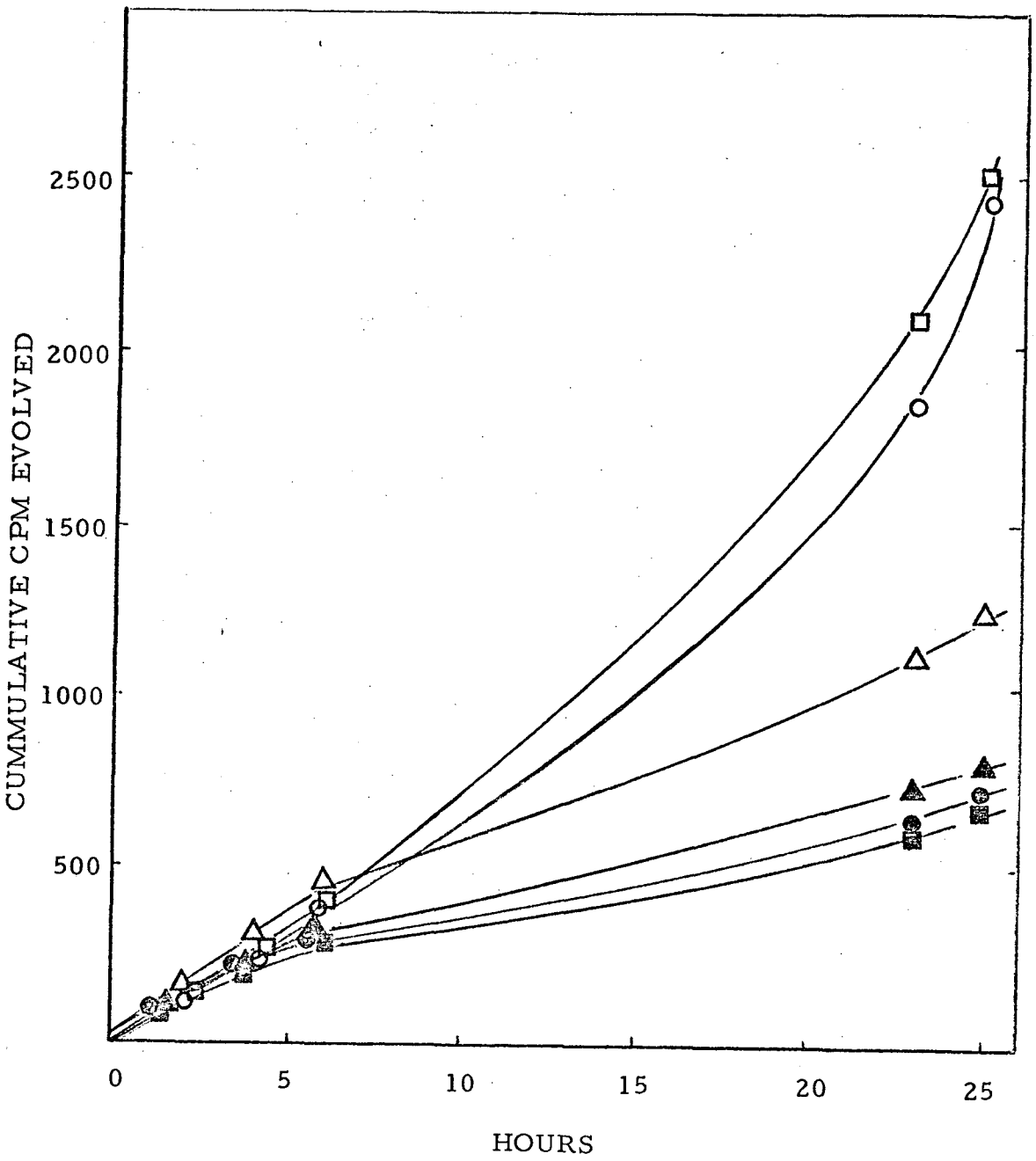


Figure 12

Effect of Ammonium Chloride on Labeled Release from Medium Containing Polyps. Two polyps were added to each medium containing 20‰ artificial sea water, ^{14}C-glucose, ^{14}C-alanine, polymyxin, and penicillin. In addition, each medium contained 0 (○——○), 3 (□——□), 10 (△——△), 30 (◎——◎), 100 (▣——▣), or 300 (▲——▲) ppm ammonium nitrogen. The evolved counts were measured over the following 25 hour incubation period.

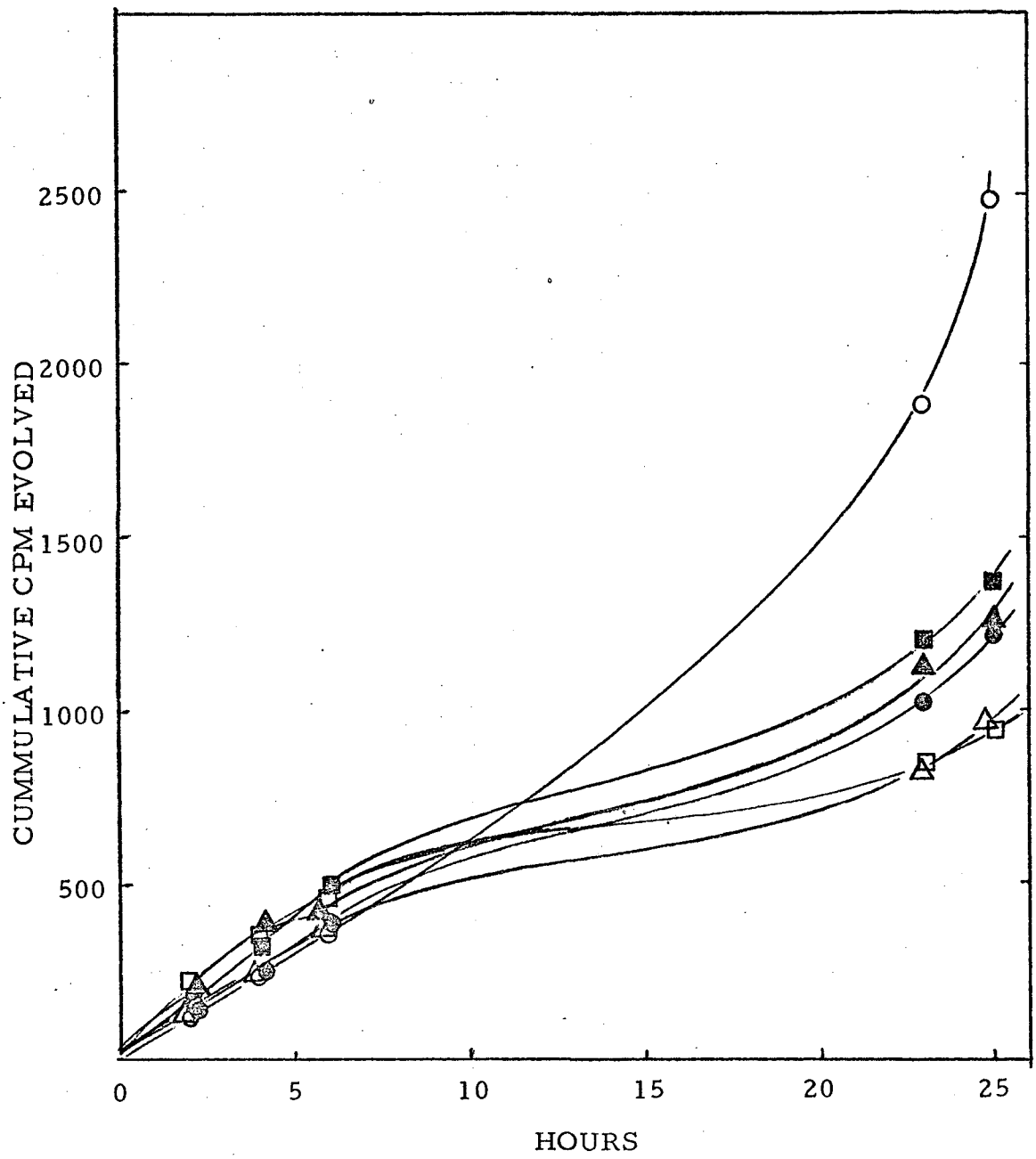


Figure 13

Effect of Phosphate on Labeled Release from Medium Containing Polyps. Two polyps were added to each medium containing 20‰ artificial sea water, ^{14}C-glucose, ^{14}C-alanine, polymyxin, and penicillin. In addition, each medium contained 0 (o—o), 0.05 (□—□), 0.25 (△—△), 1.0 (●—●), 5.0 (■—■), or 20.0 (▲—▲) ppm phosphate phosphorus. The evolved counts were measured over the following 25 hour incubation period.

the extent of inhibition is small and independent of the phosphate concentration above a certain level. The results with nitrate (Figure 14) in the range of 0 - 100 ppm nitrogen show no significant effect of nitrate at any concentration on polyp metabolism for periods up to 24 hours. These lack of significant inhibitions by either phosphate or nitrate are also in essential agreement with our maintenance studies; however, neither pollutant was observed to stimulate the metabolic rate of polyps as monitored by this technique.

One further experiment was also conducted to further explore the potential of the technique as a monitor of pollutant effects on polyps. The effects of the pollutants mercury and arsenic were examined on the metabolism of polyps in the presence of antibiotics. The results are presented in Figure 15 and show that $HgCl_2$ at 5×10^{-3}M is strongly inhibitory to polyp metabolism whereas Na_2HAsO_4 at 5×10^{-3}M is probably not inhibitory until after 20 hours of incubation. Although maintenance studies were not conducted to complement these studies, both pollutants are known to be quite toxic and are expected to be detrimental to polyp health.

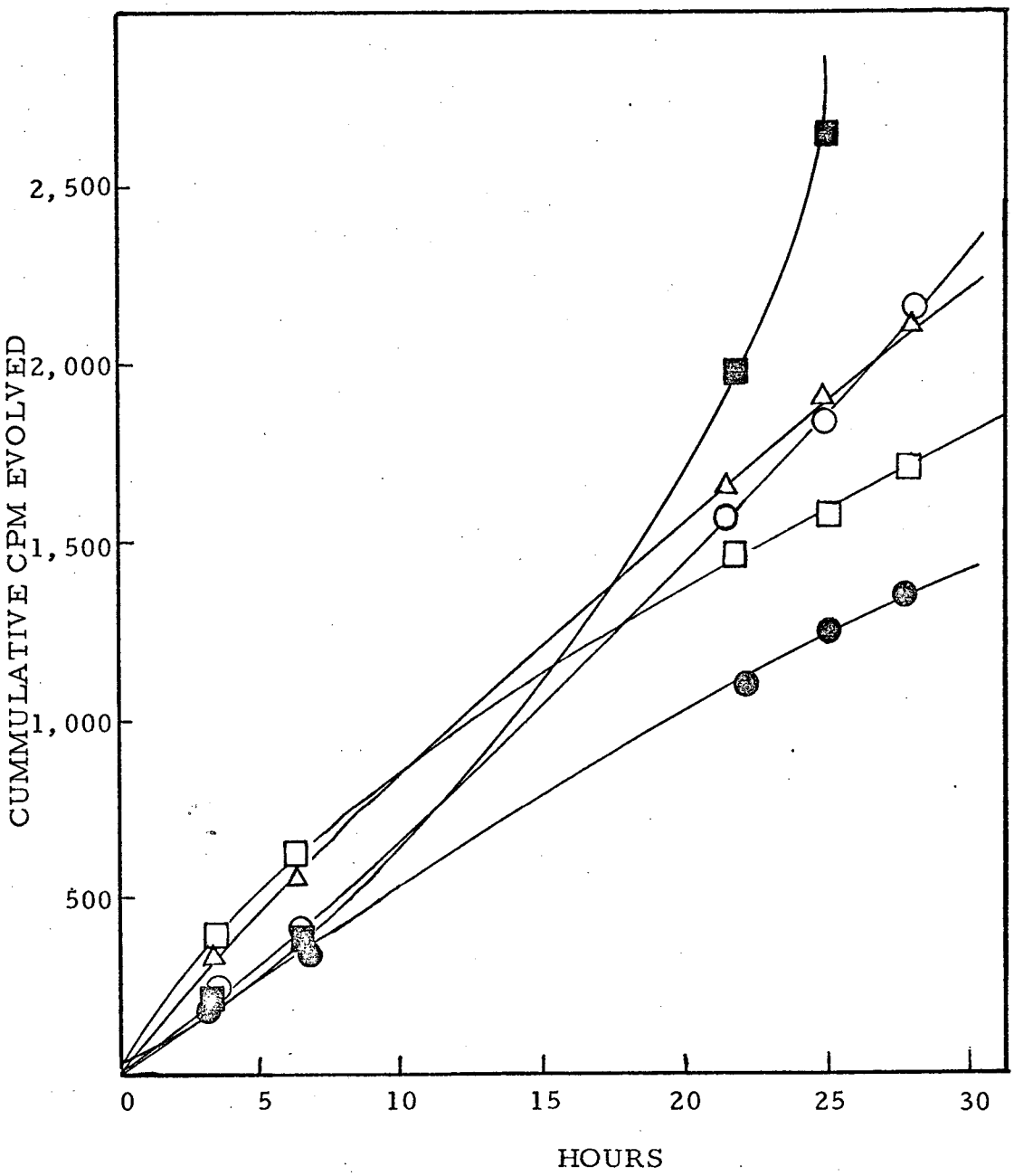


Figure 14

Effect of Nitrate on Labeled Release from Medium Containing Polyps

Two polyps were added to each medium containing 20‰ artificial sea water, ^{14}C-glucose, ^{14}C-alanine, polymyxin, and penicillin. In addition, each medium contained 0 (○—○), 3 (□—□), 10 (△—△), 30 (●—●), or 100 (■—■) ppm nitrate nitrogen. The evolved counts were measured over the following 28 hour incubation period

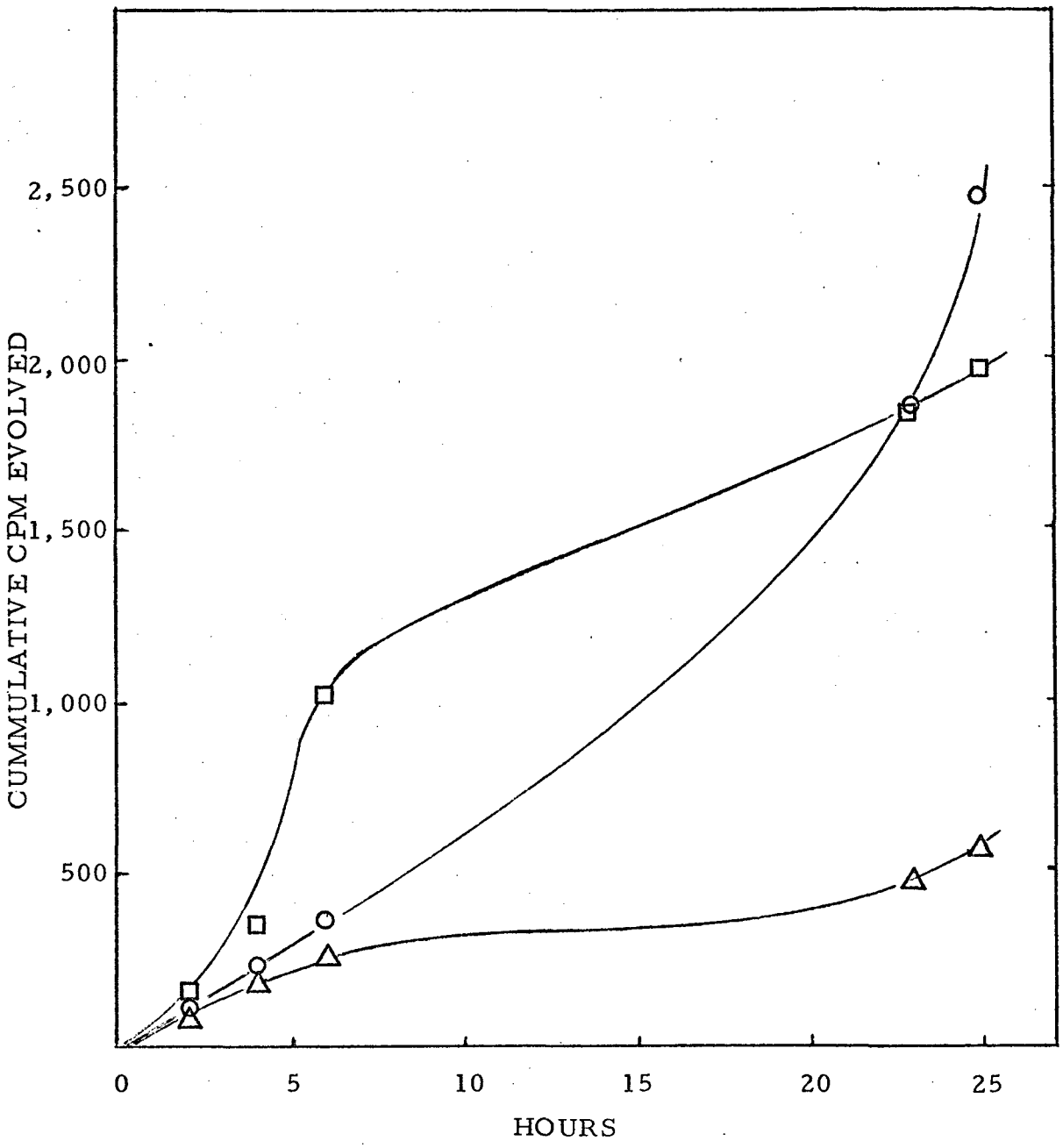


Figure 15

Effect of Mercury and Arsenic on Labeled Release from Polyps

Two polyps were added to medium containing 20‰ artificial sea water, ^{14}C-glucose, ^{14}C-alanine, polymyxin, and penicillin (○——○) and the evolved counts measured over the following 25 hour incubation period. The results are compared to reaction mixtures containing in addition either 5×10^{-3}M $HgCl_2$ (△——△) or 5×10^{-3}M Na_2HAsO_4 (□——□).

As a result of these investigations, then, it may be concluded that polyp metabolism can be discriminated from a bacterial response and monitored by the labeled release assay. Undoubtedly, the assay is applicable to other organisms as well. Using this assay, metabolic studies have been correlated with morphological effects of pollutants on polyps. The correlation is excellent for ammonium and nitrate, whereas results with phosphate were somewhat contradictory between the two measurements. Morphologically, phosphate does not adversely affect the appearance of polyps and may stimulate polyp proliferation. However, phosphate is slightly inhibitory to the metabolism of the substrates tested. For the toxic pollutants mercury and arsenic, a definite inhibition was observed. These studies suggest, then, that the technique offers a rapid screening technique, potentially useful for predetermining the effect of a given pollutant or compound on polyp morphology and survival.

V. REMOTE SENSING STUDIES

The problem of developing a technique to detect jellyfish medusae for the purpose of obtaining a census taking method is difficult from two viewpoints. First, medusae are relatively small, the largest in the Chesapeake Bay probably being about 12 inches in diameter and the average probably being about two to four inches in diameter. Isolated individuals thus are not likely to be seen at heights above 100 feet without the aid of some magnification system. However, it may be possible to overcome this disadvantage because jellyfish are often observed to occur in clusters. Thus, while individuals may not be easily detected by a particular technique, areas of high density may be detectible.

The second problem related to the detection of jellyfish is their vertical distribution in the water. They are found at various depths, perhaps depending on the time of day and the weather conditions, and also have been observed to cyclically ascend and descend in the water. These behavior patterns place certain limitations upon the remote sensing methods available for jellyfish detection. For example, the penetration of light in water is dependent on

wavelength (8). As shown in Figure 16, light at about 485 mμ (blue light) penetrates maximally whereas infrared wavelengths do not significantly penetrate water. Techniques such as infrared scanning are therefore possible only under conditions where jellyfish are present at or just beneath the surface of the water. Such conditions have often been observed on calm days when jellyfish appear to concentrate at the surface late in the morning and in the afternoon. At other times, when jellyfish are located more than a few centimeters below the surface, techniques dependent upon visible or near infrared light would be more practical.

With these considerations in mind, we have explored numerous possible techniques to establish a method for sea nettle detection. These have included reflectance, absorption, and direct photography in the visible and infrared ranges of the spectrum. Both direct and indirect methods are discussed in detail in the following sections. Of the techniques examined, detection with laser beams offers the highest promise. Also promising is the possibility of establishing a "jellyfish index" by using color infrared photography for the detection of algal species which may be associated with jellyfish blooms.

A.

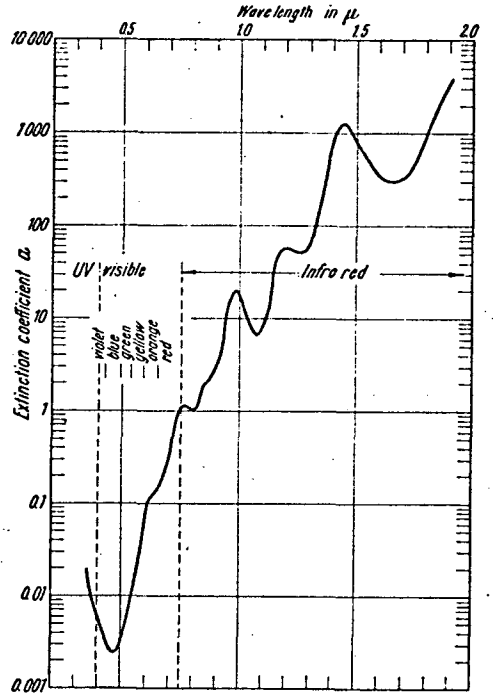


Fig. 32. Extinction coefficient a for a light beam in pure water as a function of its wavelength (thickness of layer, 1 m.). (According to G. Dietrich, 1939.)

B.

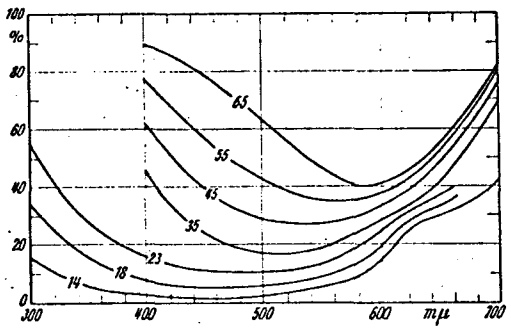


Fig. 35. Normal spectral extinction of daylight in 1 m. of sea water. Numbers on the curves indicate the average extinction in per cent per 1 m. between 388 and 721 μ. Curve 14, *extremely pure ocean water*; Curve 18, *turbid tropical-subtropical ocean water*; Curve 23, ocean water of moderate latitudes; Curves 35–65, coastal water of various degrees of turbidity (sun's elevation 90° for the first 3 cases, 45° for the remaining curves). (According to N. G. Jerlov, 1951.)

Figure 16

Water Penetration as a Function of Wavelength

Extinction of light is shown for pure water (A) and for sea water (B) from various locations. The lower the extinction coefficient, the greater would be the depth of penetration for a given wavelength (From Dietrich, Ref. 8, pgs. 76 and 81).

1. Direct Studies with Jellyfish Medusae

As a first approach to the problem of detecting jellyfish, several single lens reflex cameras were used and simultaneous photographs were taken of jellyfish in situ at various depths, from several different angles and with different films. Three color films were used for these studies: Ectachrome color film, infrared false color film, and GAF two-layer film. The latter film is a fast (ASA 1200) experimental film which has only two color sensitive layers instead of the usual three. The lack of a blue sensitive layer theoretically allows the film to "penetrate" water deeper than three-layer film. Although primarily developed for use in underwater photography, the film has recently been utilized in aerial photography over shallow water for the location of shipwrecks (19).

A comparison of pictures of sea nettles taken with each of these three films is shown in Figure 17. In terms of contrast, the greatest advantage is offered by the infrared color film where the jellyfish appear light pink against a bright blue background and the least advantage is obtained with Ectachrome film. None of these films, however, reveal

A)

B)

C)

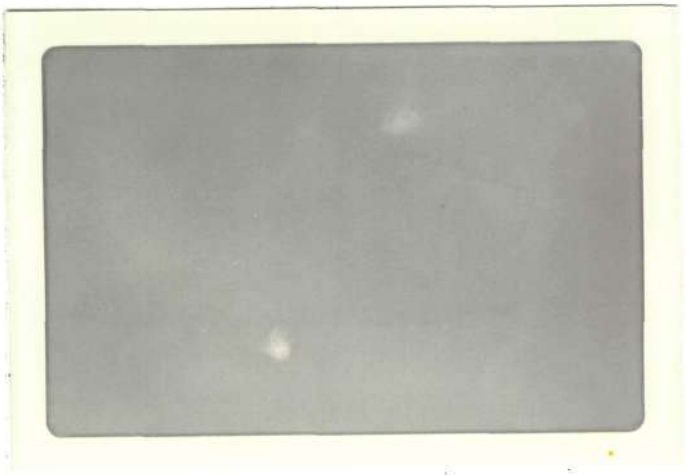
Figure 17

Jellyfish Medusae as Photographed by Three Film Types:
A Medusae floating on the water surface was photographed using A) Color Ectachrome, B) Aero Ecto Infrared (Type 8443), and C) GAF Two-layer Film.

distinguishing characteristics of jellyfish nor allow significant depth penetration. In fact, the depth penetration obtained with the GAF two-layer film is not significantly different from that obtained with the color infrared film (Figure 18). Greater depth penetration can be obtained with any of these films by utilizing a polarizing filter to remove glare from the water surface. Perhaps the best combination was obtained by combining a polarizing filter with the infrared false color film. The use of aerial photography with false color film as a detection scheme is hampered, however, by the small size of sea nettles and requires some amplification of the target area.

Our next approach to obtain a spectral signature characteristic of jellyfish was to record the infrared reflectance spectrum of a while jellyfish using a Cary 14 recording spectrophotometer against a black field. Preliminary spectra did not reveal any outstanding features that could be assigned to sea nettles. Several jellyfish were next dissected to separate the medusae bell from the tentacles. The reflectance spectra of bells, tentacles, and bay water were separately obtained and compared in the range from 0.5 - 2.4 μ. These spectra are presented in

A)



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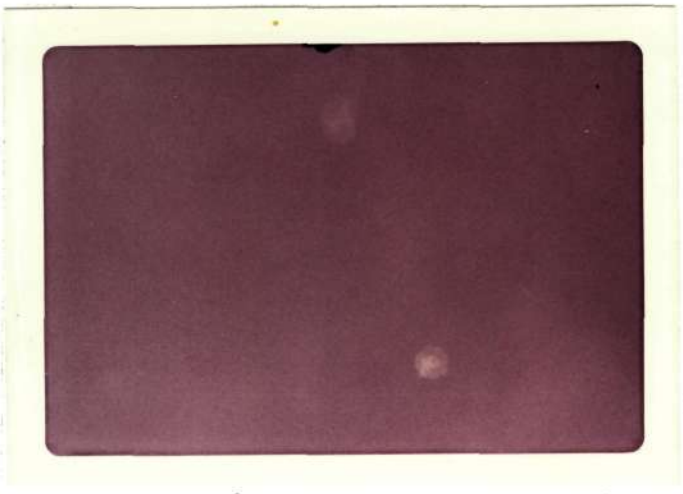


Figure 18

Comparison of Color Infrared Film and GAF-Two Layer Film for Ability to Detect Jellyfish at Two Depths: The two visible medusae were approximately ten feet from the cameras; one was at the water surface and the other approximately two feet beneath the water surface. These medusae were photographed with color infrared film Type 8443 (A) and with GAF-two layer film (B).

Figure 19. As shown, only slight differences appear among the three spectra and, again, no outstanding spectral features are apparent in this range that could represent a characteristic jellyfish signature.

To enhance the slight spectral differences observed in Figure 19, seven jellyfish were separated into bells and tentacles, pooled, and lyophilized before examination in the Cary 14. The results, presented in Figure 20, indicate a reflectance which may be attributed to jellyfish, seen in both the tentacles and bells. The larger dips in the spectra are probably due to the presence of small amounts of water in the sample since water absorption lines normally occur at 0.58, 0.78, 0.92, 0.98, 1.20, 1.45, 1.95, and above 2.0 μ.

These results, although positive, make doubtful the possibility that jellyfish can be detected in situ by a spectral reflectance signature. This is because the response is seen only with a concentrated jellyfish powder. Whole jellyfish, consisting of approximately 98 percent water, are not readily distinguishable from bay water.

Detection of jellyfish by laser beams has also been considered. This method offers the advantage of utilizing wavelengths in the visible range which is the range of

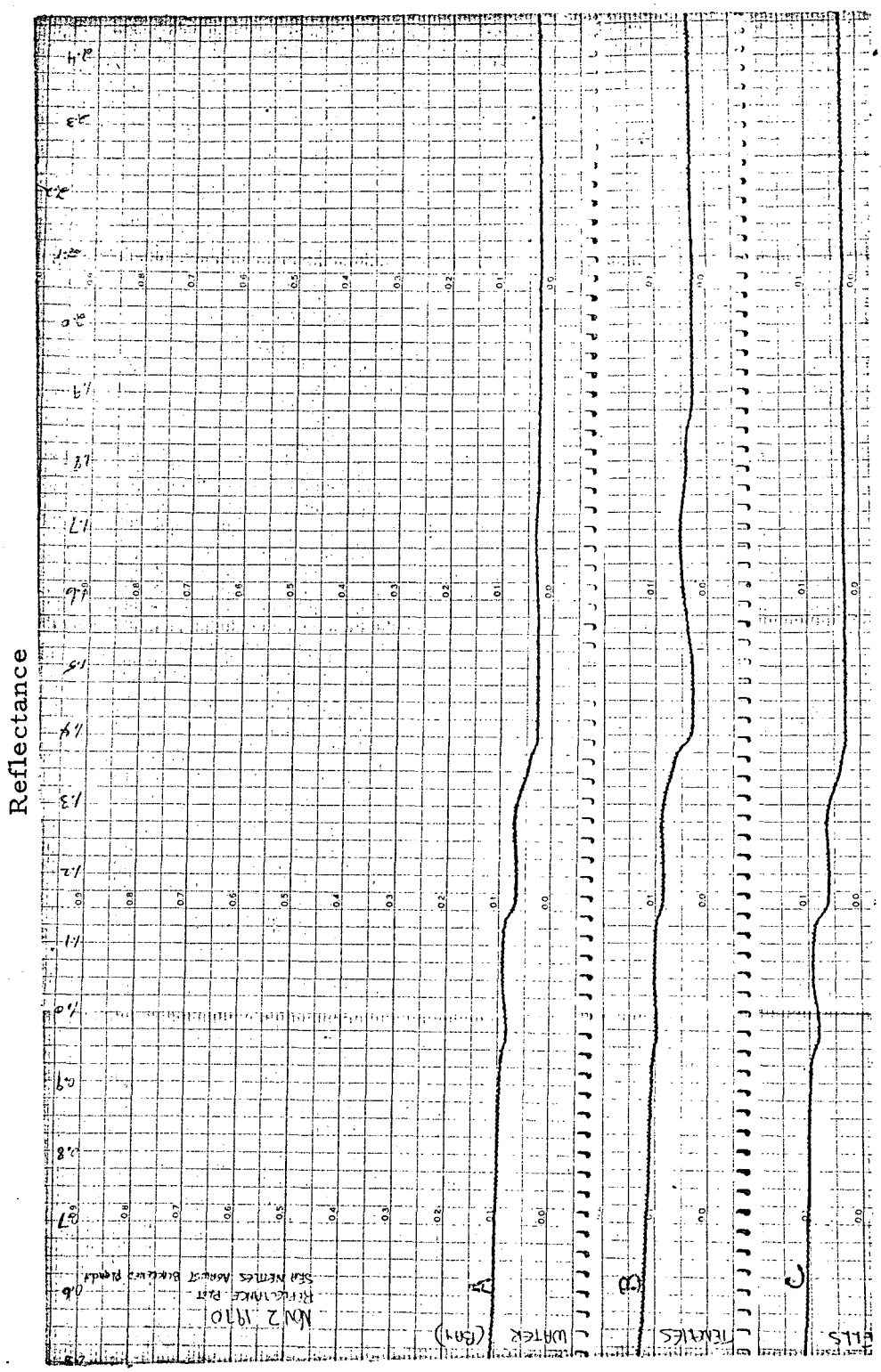
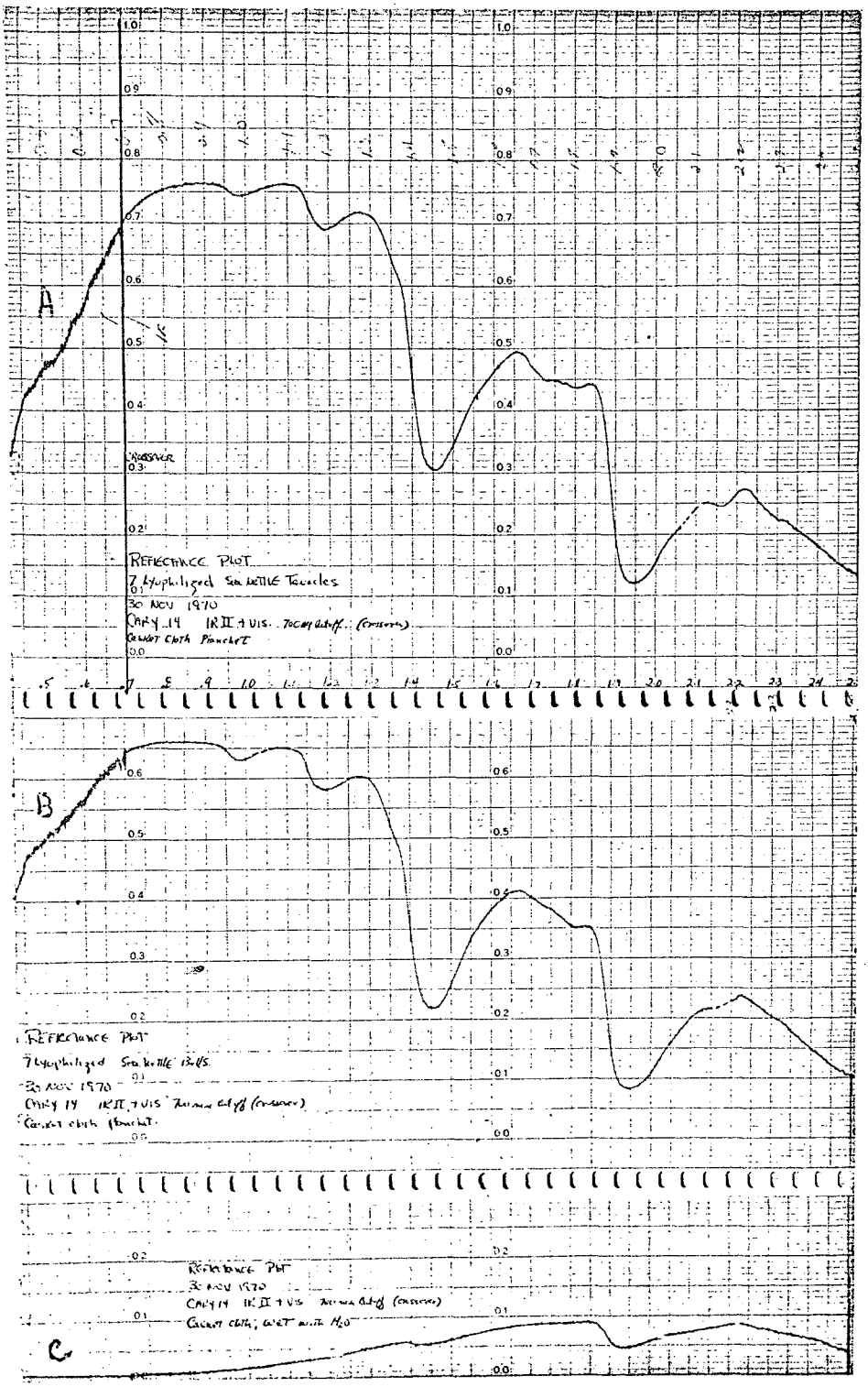


Figure 19
Comparison of reflectance from bay water (A),
sea nettle tentacles (B), and a sea nettle bell (C)
as a function of wavelength between 0.5 and 2.4 μ.
These spectra were obtained with a Cary 14 Recording Spectrophotometer.

Figure 20

Comparison of reflectance from lyophilized tentacles from seven sea nettles (A), seven lyophilized sea nettle bells (B), and bay water (C) as a function of wavelength between 0.4 and 2.5 μ. These spectra were obtained with a Cary 14 Recording Spectrophotometer



maximum water penetration. To determine the feasibility of such a detection method, we first examined jellyfish in situ in the Bay with a low power gallium arsenide laser beam at 0.6328 μ. In shallow water (less than 1 meter), the coherent light penetrated the water and was differentially reflected from the shells, sand, and mud present on the bottom. In deeper water, however, the light was absorbed before striking the bottom or being reflected out of the water. However, if a jellyfish appeared in the laser beam at one to two feet under the surface, a strong reflectance was readily observed. These findings suggested that lasers were potentially useful for the development of a jellyfish remote sensing technique assuming that jellyfish could be distinguished from other objects or life forms from which a reflectance is obtained.

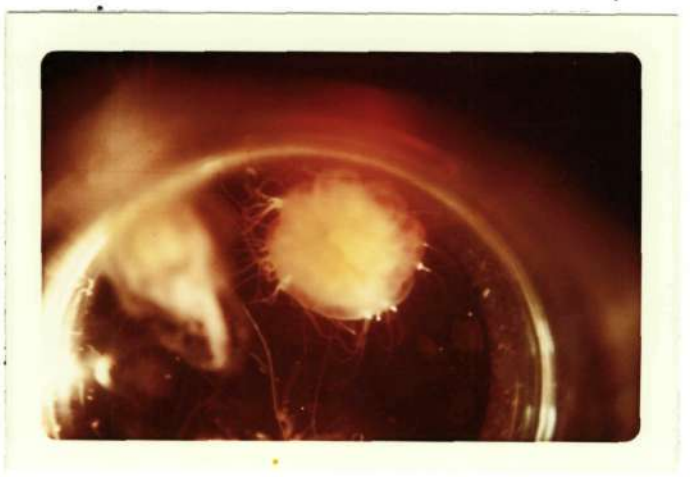
Inasmuch as Chrysaora medusae were not available during the winter months, Cyanae medusae were used for further development of the laser detection scheme. To optimize the signal, reflectances of laser beams of various wavelengths were examined. Figures 21 A, 21 B, and 21 C show the effects of illuminating a Cyanae medusae with a blue, green, and red laser beam, respectively. In each case, the jellyfish is contained within a glass beaker and

A) A jellyfish medusae of Cyanae was placed in a glass beaker and illuminated from a distance of approximately 1 meter with a laser beam of blue wavelength. This photograph was obtained from a distance of a few feet using Ectachrome Color Film. General room illumination was present.

B) A jellyfish medusae of Cyanae was placed in a glass beaker and illuminated from a distance of approximately 1 meter with a laser beam of green wavelength. This photograph was obtained from a distance of a few feet using Ectachrome Color Film. General room illumination was present.

Figure 21

Detection of Jellyfish Medusae by Laser Beams of Different Wavelengths



C) A jellyfish medusae of Cyanae was placed in a glass beaker and illuminated from a distance of approximately 1 meter with a laser beam of red wavelength. This photograph was obtained from a distance of a few feet using Ectachrome Color Film. General room illuminsation was present.

D) A jellyfish medusae of Cyanae was placed in a glass beaker and illuminated from a distance of approximately 50 meters with a laser beam of red wavelength. This photograph was obtained in a dark room illuminated only by the laser beam.

Figure 21
(continued)

Detection of Jellyfish Medusae by Laser Beams
of Different Wavelengths

the photograph was obtained in the presence of general room illumination. As shown, although each laser beam illuminates the jellyfish, the red laser beam appears to provide the best photographic contrast. The use of a red laser has certain additional advantages over green or blue light. Thus, although blue penetrates water furthest, light scatter from particles in water is also most severe with blue light. Green light, on the other hand, is readily absorbed by the organic material normally present in water.

In an attempt to maximize jellyfish detection by laser light, a red laser beam was used to illuminate a jellyfish in a beaker from a distance of 25 or 50 meters. No other light was present in the room. Figure 21 D illustrates the excellent degree of photographic contrast obtained by this method. Similar results were obtained using black and white film (Figure 22). The technique offers considerable promise for day or night detection, especially since image intensification systems are available which can attain similar resolution at altitudes of 10,000 feet.

In evaluating the feasibility of the laser beam detection method, we have examined some data regarding the turbidity of the Chesapeake Bay (20). Calculations based on these data allow the estimation that, under adverse

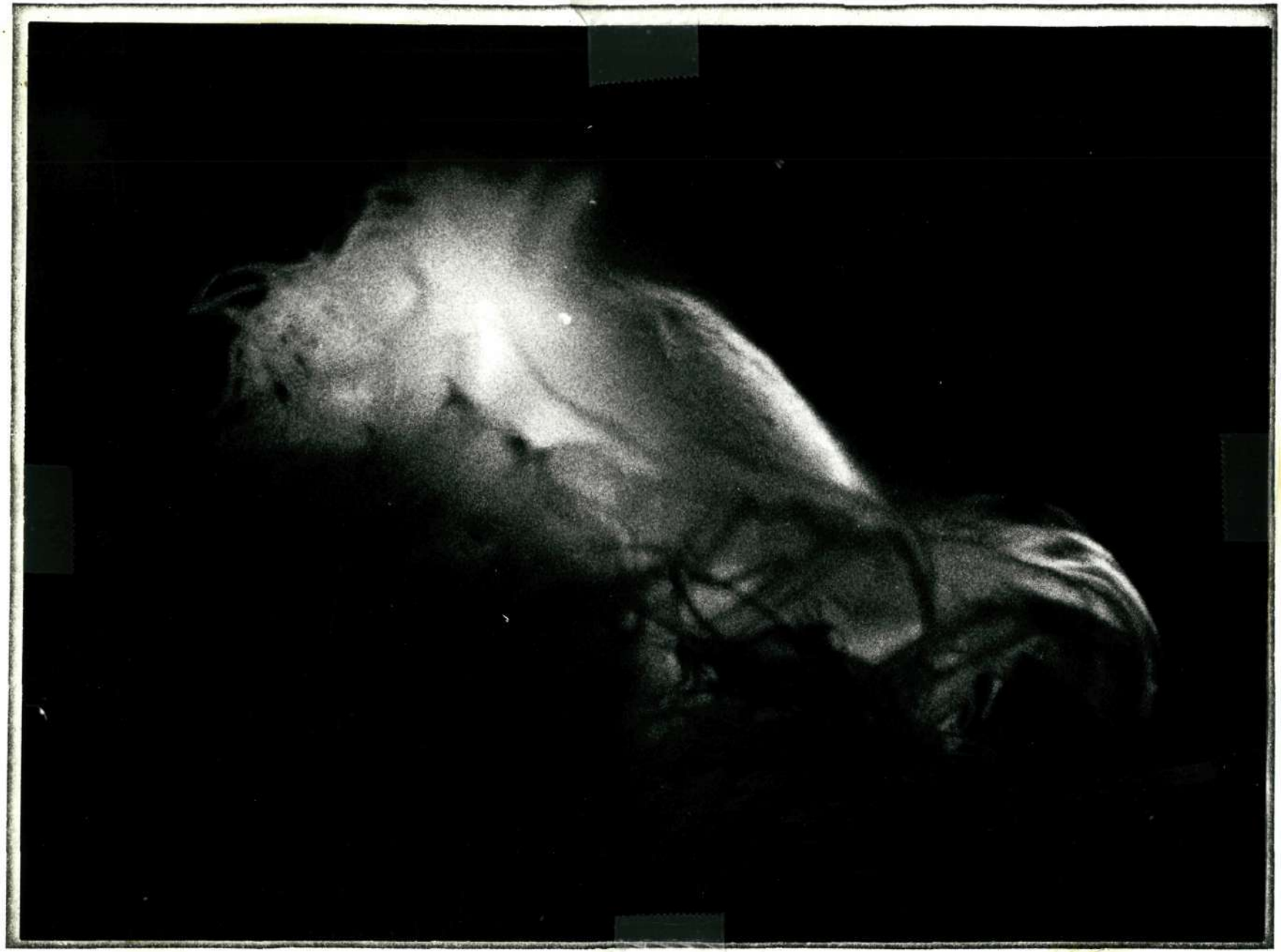


Figure 22

Chrysaora quinquecirrha illuminated by a 1.0 m. v. HE-NE Laser from a distance of 150 feet in total darkness.

turbidity conditions, maximum penetration of a laser beam would be in the vicinity of 10 meters with a 1 milliwatt laser. Since the average depth of the Bay is well below this depth, this would not pose a serious limitation for aerial surveys when conducting studies in the main channel. For an underwater detection system (see below), this calculation defines the upper limit of the sample size which could be examined by the detection system.

Utilization of the red laser beam for jellyfish detection also requires that interferring reflections be at a minimum. In field experiments, we have attempted to illuminate jellyfish at night using a red laser beam. Unfortunately, water surface reflections from ripples, waves, foam and ground swell greatly interfere with the detection of jellyfish. Thus, although jellyfish can be detected by laser beams in perfectly still water, the method per se is not practical since water is rarely sufficiently still.

Two ways appear possible in which the interferring surface reflection may be eliminated. First, laser energy could be transmitted in pulses to an underwater target. As each pulse reached the water, a portion would be reflected from the surface and the rest of the energy would

penetrate. A reflection sensor above the surface would be turned on only after the surface reflected light had returned past it. In this way, only light reflected from beneath the water surface would be detected. Such precise timing techniques have been worked out for other applications.

A second technique to eliminate surface reflection would be to operate the entire laser detection system beneath the surface of the water. For example, underwater detectors could be arranged in an "optical fence" consisting of a series of parallel laser beams on one "picket" beaming to a target on another picket. The lasers would be spaced so that any object of interest breaking the beams would induce a recognition pattern in a computer-type receiver of the output of the detectors. Information obtained from such a fence could be communicated to a surface vessel or station, or, in the ultimate application, to a periodically interrogating satellite for relay to a ground station.

Although several other approaches have also been considered for jellyfish detection, none have appeared more promising. We have been in contact with Dr. Frank S. Kennedy at the Florida Department of Natural Resources in St. Petersburg, Florida. Dr. Kennedy has been engaged for

the past year on a project regarding remote sensing of the larger Portuguese man-of-war and has kindly sent us copies of his progress reports. His efforts have also not been successful, although a variety of techniques have been examined, and he relies on low-flying aircraft for detection of surface Portuguese man-of-wars. Our attention has also been called to an article entitled "Reflecting Power of Jellyfish" (21) which appeared in a Russian journal. However, upon acquiring a translation of this article, we discovered it contained an account of sound reflection from medusae with the conclusion that echo signals from medusae can be confused with those from fish. In spite of the many problems, then, an approach using lasers holds the highest promise for the remote detection of jellyfish medusae.

2. Indirect Studies with Algae

As discussed in the preceeding section, our results have established that in photographing sea nettles, maximum contrast can be obtained with color infrared film. However, infrared wavelengths are limited in their depth of penetration and only detect sea nettles at nor near the surface of the water. The possibility exists that a "jellyfish index"

could be detected indirectly by color infrared photography if certain minerals, pollutants, or algal species which may be associated with jellyfish were detectible. Certain coelenterate species are known, for example, to exist symbiotically with certain algal species (6) although jellyfish are known to be carnivores (6).

As a first approach to the problem of indirect jellyfish detection by algae detection, we first confirmed that algae can be detected on color infrared film by a characteristic red color. To fully explore the potential and the limitations of this method of algae detection, we next examined the possibility of remotely differentiating types of algae. To this end, we acquired a variety of pure algal cultures (Department of Botany, Indiana University, Bloomington, Indiana). Our collection included two green algae (Chlorella sorokiniena and Euglena gracilis), one blue-green algae (Anacystis nidulans), and one yellow algae (the diatom Nitzschia closterium). In addition, we collected samples of brown, red, and green algae from the shore of the Chesapeake Bay and have also cultured one bacteria (Escherichia coli)for comparison. The culture media used for maintaining the various cultures are shown in Table 20 (22-24).

Table 20

Media Used to Grow Various Algal and Bacterial Cultures

CULTURE	MEDIUM CONTENTS	GRAMS/LITER	PH	°C	AERATION	REFERENCE
Anacystis nidulans (blue-green algae)	KNO_3	5.00	no adjust.	26° (room)	bubbled with air	20
	K_2HPO_4	0.10				
	$MgSO_4 \cdot 7H_2$	0.05				
	Fe Ammonium Citrate	10 drops of 1% sol'n				
	Agar	15				
Chlorella sorokiniana (green algae)	NH_4NO_3	1.00	6.8	26° (room)	bubbled with air	20
	K_2HPO_4	1.00				
	$MgSO_4 \cdot 7H_2O$	0.25				
	$Na_2 \cdot Mn \cdot EDTA$	0.0071				
	$Na_2 \cdot Ca \cdot EDTA$	0.0071				
	$Na_2 \cdot Co \cdot EDTA$	0.0077				
	$Na_2 \cdot Cu \cdot EDTA$	0.0093				
	$Na_2 \cdot Zn \cdot EDTA$	0.0067				
	$Na_2 \cdot Fe \cdot EDTA$	0.038				
	MoO_3	0.0017				
	H_3BO_4	0.010				
Escherichia coli (bacteria)	Nutrient Broth	8.0	no adjust	37°	Shake	21
Euglena gracilis (green algae)	KH_2PO_4	0.02	no adjust	26° (room)	bubbled with air	21
	K-citrate $\cdot H_2O$	0.04				
	$MgSO_4 \cdot 3H_2O$	0.02				
	Trypticase (BBL)	0.6				
	Liver L or Liver-Extract Concentrate (NBCo)	0.2				
	Agar (Oxoid ionagar #2 or #3)	12.0				
Nitzchia closterium (Diatom; yellow algae)	NaCl	5.0	7.75	18°	1-2% CO_2 in air	22
	$MgSO_4 \cdot 7H_2O$	1.2				
	$NaNO_3$	1.0				
	KCl	0.60				
	$CaCl_2$	0.30				
	K_2HPO_4	0.10				
	Tris	1.0				
	Na_2EDTA	3.0				
	H_3BO_3	0.60				
	$FeSO_4 \cdot 7H_2O$	0.20				
	$MnCl_2$	0.14				
	$ZnSO_4 \cdot 7H_2O$	0.033				
	$Co(NO_3)_2 \cdot 6H_2O$	0.0007				
	$CuSO_4 \cdot 5H_2O$	0.0002				

Considerable technical difficulties were at first encountered with the infrared film. This was traced to the fact that Kodak recently introduced a new color IR film on the market, called "Ectachrome Infrared" film. In so doing, they discontinued the color "Aero Ecto-Infrared" film (Type 8443) that has been used for most aerial photography to date. Figures 23 A and 23 B represent typical results with the newer film type. From left to right, the cultures of Figure 23 A are green algae collected from the Bay, E. gracilis (green algae), E. coli (bacteria), C. sorokiniena (green algae), and a mixture of red and brown algae also collected from the Bay. Figure 23 B pictures E. gracilis, a mixture of Bay algae, and C. sorokiniena. One interesting result is shown in Figure 23 A where, in contrast to the pure algae cultures, the pure bacterial culture has no red or purple color when photographed with color IR film. Thus, algae cultures are readily distinguishable from bacterial cultures by false color film. However, the characteristic reds and magentas usually associated with color infrared photography are not obtained with the newer film. Even plants (not shown) appeared deep purple with this film. Many attempts were made to improve these results by varying

A)

B)

Figure 23

Algae and bacteria cultures as photographed by Kodak's Ectachrome Infrared color film. Both photographs were obtained in outdoor lighting.

A) From left to right, the cultures are green Bay algae, Euglena gracilis, Escherichia coli, Chlorella sorokiniena, and red and brown Bay algae.

B) From left to right, the cultures are Euglena gracilis, green Bay algae, and Chlorella sorokiniena.

the "f" stop, the speed, and the filter in a controlled set of experiments. None of these variations produced satisfactory results despite Kodak's insistence that the film is no different than the old film (Type 8443). Following these results and conversations with other investigators, who confirmed our conclusions, we decided to continue our investigations exclusively with the Aero Ecto Infrared film (Type 8443) as long as the supply lasts.

Pictures of various pure algae cultures taken with Type 8443 film are shown in Figures 24 and 25. The three photographs in Figure 24 are essentially the same. From left to right, the cultures in the flasks represent E. gracilis, C. sorokiniena, and A. nidulans. The left petri dish contains a green Bay algae and the right petri dish contains a mixture of red and brown Bay algae. Figures 24 A and 24 B were taken out of doors with Ectachrome and Aero Ecto Infrared film, respectively. Figure 24 C was taken indoors with Type 8443 film and an electronic flash unit, demonstrating that similar results can be obtained both indoors and outdoors. Figures 25 A and 25 B were also taken indoors with Ectachrome and Aero Ecto Infrared film, respectively. From left to right, contained in the test tube cultures are N. closterium (yellow

A

B

C

Figure 24

Plant material and algae cultures photographed with Kodak's Ectachrome color film (A) and with Kodak's Aero Ecto Infrared color film (B and C). Outdoor lighting was used for (A) and (B) whereas (C) was photographed indoors with the aid of an electronic flash unit. From left to right, the flasks contain Euglena gracilis, Chlorella sorokiniena, and Anacystis nidulans. The left petri dish contains green Bay algae and the right dish contains a mixture of red and brown Bay algae.

A

B

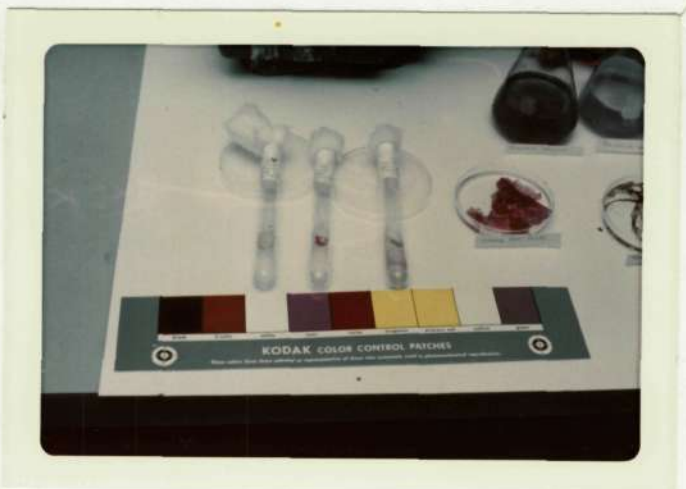


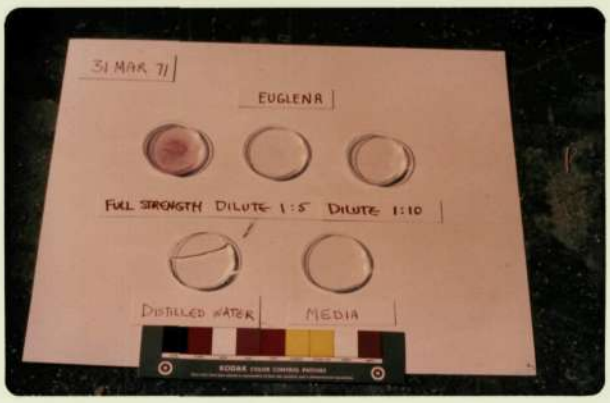
Figure 25

Algae cultures photographed with Kodak's Ectachrome color film (A) and with Kodak's Aero Ecto Infrared color film (B). Both pictures were taken indoors with the aid of an electronic flash unit. From left to right, the test tubes contain Nitzschia closterium, Anacystis nidulans, and Euglena gracilis. The contents of the flasks and petri dishes are described in Figure 24.

algae diatoms), A. nidulans (blue green algae), and E. gracilis (green algae). The contents of the Erlenmeyers and petri dishes are the same as in Figure 24. These photographs illustrate that different species of algae do have the characteristic magenta color with Type 8443 film similar to that seen for plant material and confirm the possibility of detecting algae blooms by aerial infrared photography. Close examination of the photographs in Figures 24 and 25 indicate that tone and intensity differences in the red color are evident for the various samples. These differences could reflect chlorophyll content, pigment composition, or cellular structure.

To determine the minimum number of cells detectible by this technique, different dilutions of three algal cultures were photographed with color IR film (Figure 26). Each petri dish contained approximately a 35 ml volume. The cell concentration of each full strength culture was determined with a hemacytometer (Table 21). The color of each full strength culture was readily detectible by eye and by color IR film. After dilution of each culture 5- or 10-fold, however, the algal cultures are just barely

A)



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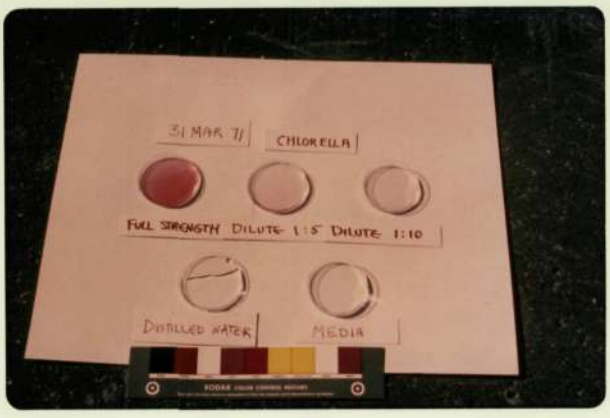

C)

Figure 26

Detection of Dilutions of Three Algal Cultures by Color Infrared Film. Aero Ecto Infrared Film was used to photograph pure cultures of Euglena (A), Chlorella (B), and Anacystis (C). Approximately 35 ml of each concentrated culture was placed in a petri dish against a white background. Each culture was also diluted 5- and 10-fold with culture medium for comparison. The cell count of each concentrated culture is shown in Table 2. Distilled water and medium alone were also photographed in each case.

Table 21

Cellular Concentrations of Algal Cultures
Pictured in Figure 26

Undiluted Culture	Cells/ml	Approximate Cells/Dish
Euglena	1.7×10^{6}	6×10^{7}
Chlorella	4.2×10^{7}	1×10^{9}
Anacystis	1.2×10^{8}	4×10^{9}

Cell concentrations of the "full strength" algal cultures shown in Figure 26 were obtained with a Neubauer-Levy ultra plane hemacytometer on a 4×10^{-6} mm^{3} volume.

detectible by eye or IR photography. That any color resulting after dilution is not caused by the medium itself is also indicated. It appears, then, that in order to detect algal blooms aerially, the cellular concentration must be in the range of 10^6 or 10^7 cells/ml. Although such concentrations are readily detected by eye, color infrared film has the advantage of offering maximum color contrast and an impartial record.

The mechanism by which the infrared light is reflected from chlorophyll-containing materials has been a subject of controversy and has been attributed both to cellular structure and to chlorophyll itself. To examine this issue, we have extracted chlorophyll from spinach leaves according to standard procedures (25). If chlorophyll reflection is responsible for its color, then the color should be apparent when photographed against either a white or black background. If, on the other hand, the color of chlorophyll results strictly from absorption properties, then it would appear red only against a white background. The results of Figure 27 show that only against a white background does chlorophyll appear red with color IR film. Therefore, colors other than those attributed to chlorophyll reflectance must be responsible for the red color obtained from chlorophyll-containing materials.

Figure 27

Chlorophyll as Photographed with Aero Ecto Infrared Film. Dilutions of a chlorophyll extract were placed in petri dishes. Half of the petri dish was then placed against a white background while the other half was placed against a black background. The black background appears red with the IR film. The concentrated chlorophyll extract contained 5.4 optical density units at 660 mμ/ml.

Chlorophyll color against a white background closely resembles that obtained from algal cultures. Thus, the algal reflectance spectra must represent a composite of both chlorophyll absorption and reflection from structures such as cellular substructures. Chlorophyll within an algal cell, by virtue of its absorption properties, acts as in interference filter and shapes the spectral reflectance characteristics of the cells in which it resides. It would be expected, then, that since various algal cultures possess different cellular structures and contain additional pigments with different absorption properties, that spectral reflectance signatures would vary for different algae.

This hypothesis has been tested by examining spectral signatures of two different algae cultures on the Cary 14 recording spectrophotometer. Separate suspensions of Chlorella sorokiniena (green algae) and Anacystis nidulans (blue green algae) were concentrated by centrifugation and placed on a glass planchet at a depth of approximately 3 mm. Spectra were then obtained against either a white MgO background or a black casket cloth background. As shown in Figure 28, the spectral signatures differ for these two algae. This is expected since they contain different pigments (26)

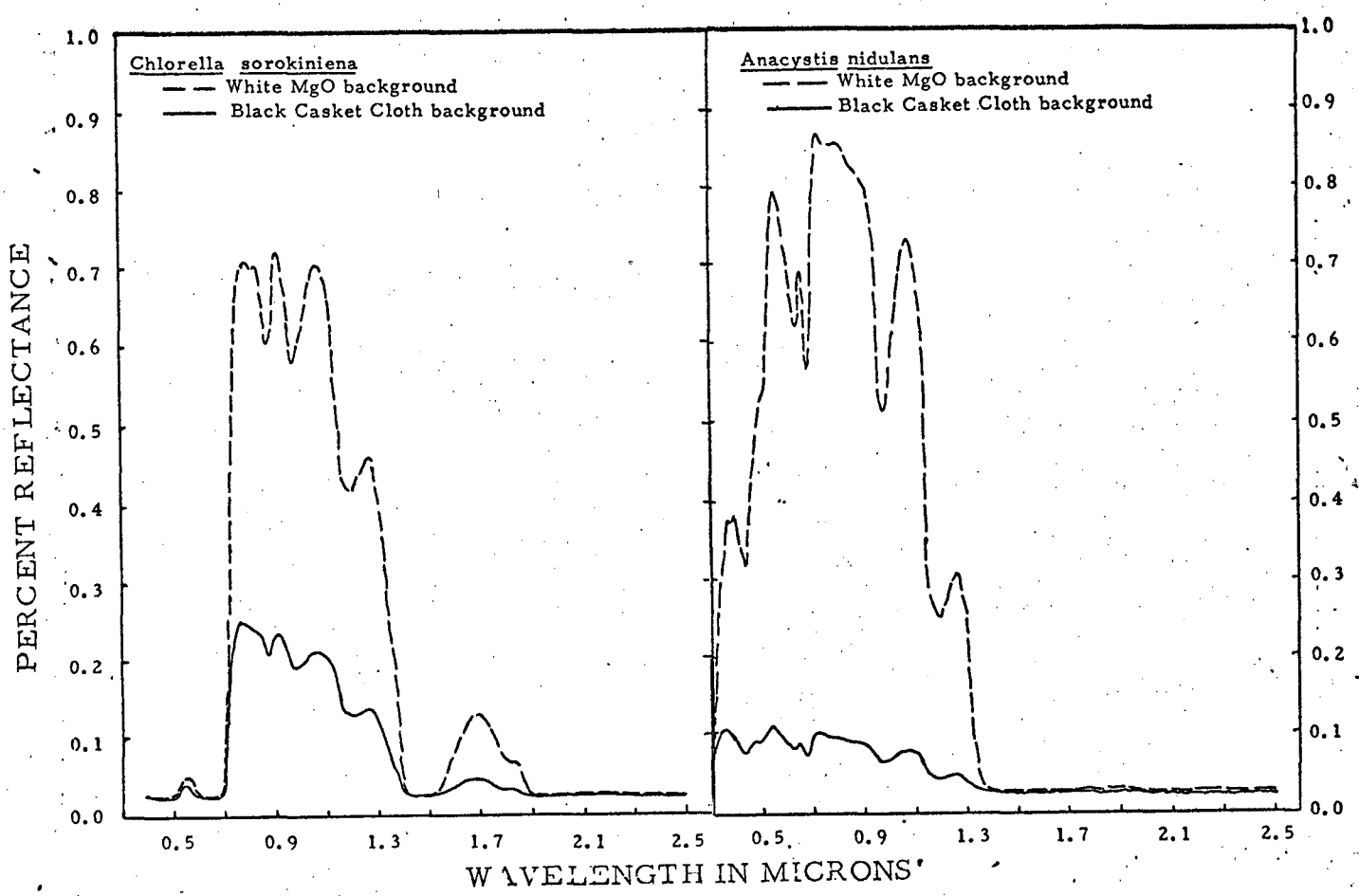

Figure 28

Reflectance Spectra of Chlorella sorokiniena and Anacystis nidulans. Algal cultures were concentrated by centrifugation and placed in a glass planchet at a depth of approximately 3 mm. Algal spectra were obtained with a Cary 14 Recording Spectrophotometer against a background of either white MgO or Black Casket Cloth, as indicated.

with different spectral characteristics and are of different cellular structure. Further, the spectrum of each algae is strongly attenuated against the white background, confirming the concept that these spectra are not reflectance spectra. Rather, they must represent a composite return of light reflected from the background which has been shaped by the absorption characteristics of the algal pigments and cellular structure. Similar results are obtained when these same algal cultures and a chlorophyll extract are examined by color infrared photography (Figures 27, 29).

In summary, these experiments demonstrate the feasibility of algal bloom detection by aerial false color photography. In addition, the experiments suggest that it may be possible to differentiate remotely between types of algae (i.e., green versus blue green) by measuring ratios of reflectance at three wavelengths. This type of detection takes advantage of the different pigments contained in the different algal types and the consequent effect of the spectral properties of algae. It is also possible that such a scanning procedure, in addition to offering a more refined discrimination, may be more sensitive than false color photography. If then an

A)

B)

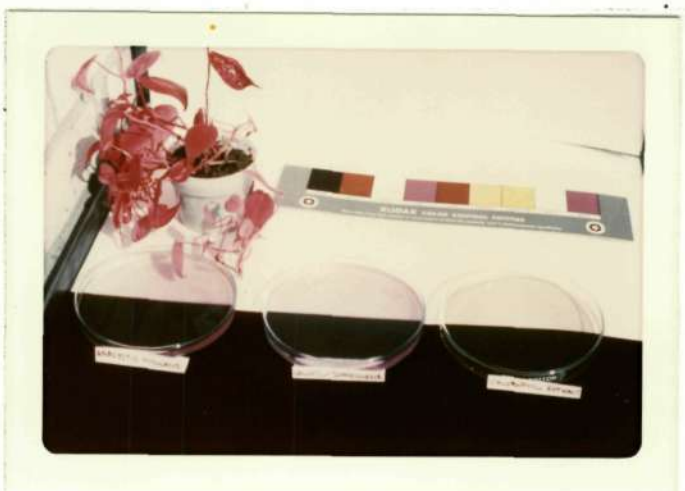

Figure 29

Algae Cultures and Chlorophyll Extract as Photographed with Kodak's Ectachrome Color Film (A), and with Aero Ecto Infrared Film (B). From left to right, the petri dishes contain Anacystis nidulans, Chlorella sorokiniena, and chlorophyll extract. Half of each petri dish was placed against a white background and the other half against a black background (casket cloth).

association between certain algal species and jellyfish could be established by field investigations, algae detection could provide an indirect method for the remote sensing of jellyfish.

VI. CONCLUSIONS AND RECOMMENDATIONS

Our investigations regarding the sea nettle Chrysaora quinquecirrha have encompassed problems related to the effects of pollutants on the polyp stage of the sea nettle and to the development of a census taking method for sea nettle medusae.

Regarding the possible role of pollutants on sea nettles, our data suggest that wastes containing phosphate and nitrate may contribute to the prevalence of sea nettles in the Chesapeake Bay. The phenomenon is temperature related and the higher the water temperature, the greater the polyp multiplicity. On the other hand, ammonium and synthetic sewage effluent are detrimental to polyps. Combinations of ammonium plus phosphate plus nitrate are also detrimental although phosphate and nitrate afford some protection against the lethal effect of ammonium. Related to these findings is the fact that polyps are responsible for ammonium disappearance from medium although they do not significantly alter the phosphate and nitrate concentrations.

These data regarding morphological effects of pollutants has been well complemented by metabolic studies with these same pollutants. Thus, in our labeled

release assay, ammonium is strongly inhibitory whereas nitrate has little effect on polyp metabolism. Phosphate is slightly inhibitory although the extent of inhibition is independent of concentration within the range studied. Nonetheless, the metabolic results are essentially in agreement with the morphological studies.

It appears, then, that a combination of morphological and metabolic studies can potentially provide valuable information related to the role of various factors on sea nettle polyps and it is highly recommended that these studies be continued. Thus, it would be of considerable value to test the effects of several other pollutants on polyps. Such a study would provide a greater understanding of the relationship between sea nettle numbers and a variety of environmental factors. In view of the large recreational and economical impact of sea nettles on the Bay, such information would be invaluable for any attempt to control them. Perhaps such data would also indicate a potential usefulness of polyps as indicators of types and amounts of pollutants. Further correlations between the metabolic and morphological methods of monitoring could also be obtained, perhaps establishing the metabolic assay as a

rapid means of predetermining the effect of a given pollutant.

For future studies, however, it is also recommended that some effort be made to obtain a homogeneous, synchronous polyp population. In our current study, the asynchrony of our population led to variable results even within the same environmental situation or among duplicate environments. Thus, while in some cases phosphate caused rapid proliferation of polyps, it did not in other cases. The causes of this variability are unknown. If this type of problem could be conquered by obtaining synchronous populations, more meaningful data could be obtained with less effort and the effects of pollutants more meaningfully determined. The application of the labeled release technique to other macroorganisms, free-swimming and benthic, may provide a valuable, new bioassay technique for monitoring and studying effects of pollutants and environmental conditions.

Regarding our remote sensing studies of sea nettles, our investigations have opened three possible means by which a sea nettle census could be taken. The first is based on the fact that sea nettles can be detected by the use of lasers. This is a method potentially useful for detecting a variety of aquatic species and offers the

advantage of utilizing wavelengths in the visible range which is the range of maximum water penetration. Further studies are required, however, to determine the specificity of the response and the differential reflectivity of sea nettles, other marine species, and objects found in the Chesapeake Bay. If many interfering responses are obtained, it is possible that sea nettle detection may ultimately require an array of multiband lasers. Full examination of the limitations imposed by water depth, turbidity, and surface reflectivity should also be included in this study.

A second possible means for jellyfish detection also involves the use of lasers. This system, however, utilizes underwater lasers arranged in an "optical fence." Jellyfish passing in the laser beams would break the beams in a characteristic manner, producing a recognition pattern in the receiver of the detector output. This method also requires examination of specificity and limitations.

The third method is based on the possibility that a "jellyfish index" could be indirectly determined if certain minerals, pollutants, or algal species which are associated with jellyfish were detectible. In our study,

algae can be readily detected by color infrared film. Although this method is not particularly sensitive, it may be possible both to differentiate algal types (green versus blue-green) and to increase the sensitivity by taking spectral scans and determining ratios of reflectance at different wavelengths. A correlation of algae to jellyfish would then allow indirect sea nettle detection.

Since each of these three methods offers promise for sea nettle detection, it is recommended that all three be simultaneously pursued. Perhaps even a combination of all three methods would ultimately make detection of sea nettles, as well as other marine species, possible.

Respectfully submitted,

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